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Keynote Lectures

KL01

Role of FNAB in the Developed and Developing World: Current Challenges

Andrew S. Field

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FNAB is an excellent, minimally invasive and accurate tool for the diagnosis of palpable and impalpable, superficial and deep seated benign and malignant lesions. In the developed world, EUS and EBUS have further increased the diagnostic role of FNAB. In the era of personalized medicine and specific treatment of tumors, FNAB in most cases can provide the material for the most sophisticated molecular studies. Current challenges include both the most basic pre analytical problems with the actual performance of the FNAB and the making of direct smears, and the requirements for increasing analytical accuracy, the use of structured reporting and the triaging and preparation of material at the time of the FNAB for ancillary testing. In the developing world, where FNAB can offer great benefits in the diagnosis of infectious and tumorous lesions, the great challenge is to increase the number of trained cytopathologists and cytotechnologists and to support them in developing their practices and the use of FNAB cytology. There have been a number of initiatives to assist this expansion of FNAB services and one will be briefly reviewed. Possible future programs to increase the teaching of FNAB cytology will be presented.

Disclosure of Interest: None declared.

KL02

Cytology and Molecular Diagnosis in Lung Cancer

Dong-ge Liu

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Approximately 70% of lung cancers are inoperable and at an advanced stage when patients come to the hospital, in which case cytology may be the only available sample to make a diagnosis. Gene target therapy needs to be based on an accurate diagnosis, which can extend the survival of late stage patients with a quality life and less side effects as compared to chemo therapy. More than 50% of cytology samples can be diagnosed based on morphology alone, while the rest may need ancillary test of immunohistochemistry and special staining. In this talk we will share our experiences of cytology diagnosis of lung cancer in an algorithmic approach spanning samples of effusion, sputum, bronchial lavage and brushings, EBUS TBNA and FNA samples. We put special emphasis on body effusion, through which we diagnosed 316 cases of lung cancer of adenocarcinoma, 55 small cell lung cancer and 36 squamous cell carcinoma over 1000 cases by applying panels of IHC markers on cell blocks in the last 5 years. Molecular test (AmoyDx, Xia Men, China) was also applied to cytology samples after accurate diagnosis of lung adenocarcinoma, where mutation rate of EGFR was 47.8%, gene fusions of ALK 9.0%, RET 3.7% and Ros-1 2.2%.

Disclosure of Interest: None declared.

KL03

Cytology Services in the Middle Income Trapped Country: Thailand Experiences

Samreung Rangdaeng

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Middle income countries (MICs) are defined by World Bank as having per capita gross national income of US$ 1,026 to $12,475. They represent 5 of 7 billion world population and 73% of world's poor people. Diversification of size, population and income level, each country has different financial and human resources to provide decent healthcare to its own population. While advanced medical technology are available in these countries which are usually limited to those who can afford, balancing between providing adequate health care services and limited resource is a unique challenge each country need to face. Cytology services depend heavily on skill of well-trained personnel rather than advanced technology. Hence, it serves as a bridge between conventional way of practice and technology.

During the past decade in Thailand, cytology services had played a major role in the prevention and management of two leading cancers in women i.e. cervical and breast cancers. Approximately two million conventional Pap smears were performed each year according to second 5-year phase of nationwide cervical cancer screening program organized by National Cancer Institute of Thailand. Fine needle aspiration biopsy of the breast was the primary workup choice to investigate women with breast lumps or those with abnormal mammographic findings.

Thai Society of Cytology, inaugurated in 2000, has taken part of this national drive in aspects of quality assurance, proficiency testing, human resource development and cytology education. Collaboration with global community of cytology has fostered academic atmosphere and improved overall standard of cytology practice for the country.

Disclosure of Interest: None declared.

KL04

Role of the Pathologist in the Management of Breast Cancer

Fernando Schmitt

Laboratoire National De Santé, Luxembourg

Breast cancer has become a major health problem globally, being the most common female cancer in many parts of the world. The main role of the pathologist in the management of breast cancer is to provide and accurate diagnosis and reporting the baseline expression of prognostic and predictive biomarkers to guide clinicians for optimal therapeutic strategies. The diagnosis of breast cancer is morphological, so the interpretation of histological or cytological images are still the gold standard for the diagnosis and only after a pathological diagnosis a patient can be treated definitively. Pathologists are essentialin the evaluation of efficacy of surgical treatment during intraoperative and post-operative periods; deciding the appropriate tissue submission, evaluating surgical margins, and ensuring the appropriate sentinellymph node mapping. Moreover, pure morphological data as size of the tumor, typing, grading, and lymph node involvement are still the most important prognostic factors in breast cancer. Our increased understanding of the underlying molecular/genetic mechanisms of breast carcinogenesis has led to the widespread interest in the molecular classification of breast cancer, which is very useful for the management of the patients. In the daily practice the use of markers such as hormonal receptors (ER/PR), HER2 and the proliferative index (Ki67) allows a correct choice of the therapy for most of the patients, although for some only molecular signatures can provide better stratification. At present, cytology still has an important role in the management of breast cancer. Using cytology we can assess pre-operatively the axillary lymph nodes, improve the performance of core biopsies enabling on-site assessment and deciding the requirement of further passes. Fine needle aspiration (FNA) also can be used to identify multicentricity or multifocality of the primary breast carcinoma when surgeons consider breast-conserving surgery. Another important field to use FNA material is for the molecular assessment of metastatic breast cancer, which is usually diagnosed by a combination of clinical and imaging findings. Once diagnosed the choice of systemic therapy is based on the characteristics of the primary tumor and is well demonstrated that intra-tumor heterogeneity is not uncommon in breast cancer. Many studies show discrepancies between markers in primary tumors and their respective metastases highlighting the need to obtain tissue confirmation of recurrences in breast cancer. FNA is a safe, trustworthy and cheaper alternative to surgical biopsy to obtain material from metastatic sites to study biomarkers and perform genetic profiling. In summary, this lecture addresses the participation of the pathologist in all major steps in the management of breast cancer patients not only as isolated morphologists but also as 'diagnostic oncologists' in multidisciplinary approach.

Disclosure of Interest: None declared.

Presidential Guest Lectures

PGL02

The Biology and Clinical Significance of HPV

John Doorbar

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Human papillomaviruses are a diverse group of viruses that cause epithelial lesions with different characteristics and with different cancer associations. Because these viruses have co-evolved with humans for millions of years, many cause only subclinical infections. The Beta HPV types that typically infect cutaneous skin are examples of such viruses. By contrast, many Alpha HPV types are associated with more problematic disease, including the recalcitrant genital and laryngeal papillomas caused by 'low-risk' types, and also the neoplasias and cancers caused by 'high-risk' papillomaviruses.

High-risk HPV types have a different life-cycle organisation from the low-risk papillomavirus types, which underlies their increased association with cancer. All papillomaviruses establish their viral genomes as low copy number episomes in cells of the epithelial basal layer. In contrast to the low-risk HPV types however, which show only low levels of viral gene expression in infected basal cells, high-risk papillomaviruses can clearly drive cell proliferation in these cells, with elevation of E6 and E7 gene expression underlying the development of HPV-associated neoplasia. Thus while both high and low-risk HPV types require E6 and E7 to drive cell cycle entry for genome amplification, only the high-risk HPV types use these gene products to cause cell proliferation in the basal and parabasal layers. The deregulated expression of these genes, as occurs in high-grade neoplasia, leads to the accumulation of chromosomal abnormalities and mutations in the host genome, as well as epigenetic changes that are required for neoplasitic progression.

The deregulation of high-risk viral gene expression occurs at particular epithelial sites. The best characterised of these is the cervical transformation zone and endocervix, where the majority of cervical cancers arise. A similar situation is found at the anal transformation zone, with other transformation zone regions in the body likely be sites of deregulation upon infection. A similar line of thinking is also thought to explain the association of high-risk HPV infections with oropharyngeal cancers developing from infections of the tonsillar crypts.

Although persistent infection can lead to cancer, in most cases, HPV infections are resolved as a result of a host immune response, leading to clearance or to viral persistent as a latent infection. Latency and reactivation as microlesions, may account for the detection of HPV DNA in the absence of disease in some in 5% or so of women. These results prompt us to consider life-cycle biomarkers, and markers of active viral infection as superior tools for the stratification of HPV-associated cervical neoplasia, when compared to pathology analysis alone, or the use of HPV testing.

Disclosure of Interest: None declared.

IAC Award Lectures

IA01-2

[Kasumas Masubuchi Award 2014] Population-Based Screening for Uterine Cervical Cancer by TOBO Method – Cooperated System by Administrative District Unit, Members of Tokyo Association of Obstricians and Gynecologists

Toshihiko Hasegawa

The Tokyo Health Service Association, Japan

The Tokyo Metropolitan Government started its screening program in 1968, using a population-based screening model for urban areas. This method is called the 'TOBO method,' in which the local government authorities responsible for the screening services (ward/city/town/village) carry out the screening by concluding contracts with the local branches of the Tokyo Medical Association and the Tokyo Health Service Association. More specifically, examinees visit member facilities of the Tokyo Association of OB/GY and provide cell samples, which are then sent to the Cytology Center of Tokyo Health Service Association. The assessment results are notified to the member facilities and local government authorities. If any abnormality is detected in the cytology, the physician explains the results to the examinee, and detailed examinations are performed. The member facility notifies the results of the detailed examinations, in principle, to the local government authorities and the Tokyo Health Service Association, and the data are tabulated.

The number of specimens increased gradually to 43552 in 1977. With enactment of the Law of Health and Medical Services for the Elderly in 1982, followed by the start of the cancer screening project subsidized by the government under the law in 1983, the number of screening examinees increased and reached 200000 by 1993. Thereafter, due to the impact of the phrase 'Cancer screening does nothing but harm' put forwarded by a radiologist, which was then picked up by a leading mass media company, and also the impact of the replacement of the special government subsidies for cancer screening with general revenue resources in 1995, the number of specimens decreased gradually to a little over 170000 by 1999. The guidelines for uterine cervical cancer screening in Japan were revised in 2004. In the revision, the recommended frequency of screening was changed from 'every year from 30 years of age' to 'every 2 years from 20 years of age.' Due to this change, the number of specimens decreased further to under 170,000. In 2009, 'Women-specific cancer screening promotion project' was taken up as a national policy. Free coupons for screening were distributed to women of specific ages. Due to the increase in the number of examinees using the free coupons, the number increased and has remained since then around 240,000. The notable feature is that the target group which was women who are 30 years or older, was changed to women who are 20 years or older. As a result, also helped by the free coupons, the number of examinees aged less than 30 years has increased greatly.

Analysis of the results will be discussed.

Disclosure of Interest: None declared.

IA01-3

[International Cytotechnologist of the Year Award 2014] Thyroid Cancer Is the Highest Incidence of Cancer in Korean Women

Jong Yull Kim

Eulji University, Korea

According to 2014 KCCR (Korea central cancer registry) data, common types of cancers among the Korean women were thyroid cancer, breast cancer, colon cancer, stomach cancer, lung cancer, and liver cancer in regular order.

In 2001, thyroid cancer was ranked only 7th, but it became the number one most common cancer since 2005 to 2014.

The reason for increased portion of the incidence rate of thyroid cancer is probably due to increasing availability of public health checks, development of ultrasonogram, and good-technique of FNABC as there's no definite other evidences to probe that type.

FNABC technique using especially ultrasonogram & immunochemistry staining was attributed to well marked cytologic smear pattern.

According to study conducted by Cell & tech bio institute in 2014, papillary carcinoma of thyroid cancer among the Korean women accounted for 83 percent of the entire.

The rest are follicular cancer (7%), followed by medullary cancer (4%), malignant lymphoma (3%), anaplastic cancer (2%), and others (1%). Cytologic features of each main diagnoses are as follows.

Grave's disease yields specific cellular features consisting of glandular ring pattern, marginal vacuoles, and tall follicular cells.

Subacute thyroiditis yields lymphocytes, plasma cells, epithelioid cells, and MGH. Langhans giant cells are clear features of subacute thyroiditis.

Hashimoto's disease yields abundant lymphocytes, Hürthle cells, plasma cells, pseudofiber, and basket cells. Lymphocytes and Hürthle cells are clear features of Hashimoto's disease.

Papillary carcinoma is the most common malignant tumor of thyroid gland. Needle aspiration yields true papillae, psammmoma bodies, and atypical nuclei including intranuclear pseudo-inclusions & grooves.

Follicular carcinoma yields atypical follicular cells which appear as isolated cells as well as clusters. Microfollicles with nuclear atypism are clear features of follicular carcinoma.

Medullary carcinoma are solid well-demarcated, but not encapsulated.

Needle aspiration yields tumor cells which appear as small sheets or clusters as well as individual spindle cells.

Stromal amyloid appears as spherical or rod-shaped homogeneous material that stains amphiphilic with the Pap., method, Congo red is more specific as it stains red color. Spindle tumor cells with stromal amyloid are clear features of medullary carcinoma.

Malignant lymphoma has been discussed with differentiation from Hashimoto's disease.

Needle aspiration yields abundant one cell pattern of lymphoid cells as well as nuclear atypism.

Atypical lymphoid cells with one cell pattern are clear features of malignant lymphoma.

Anaplastic carcinomas including small & large cell type are thyroid cancer that sometimes occur.

Unlike malignant lymphoma, anaplastic small cell carcinoma shows clustering, molding, salt & papper chromatin pattern, or an abundance of oval cells.

Disclosure of Interest: None declared.

IA02-1

[Maurice Goldblatt Award 2015] Exciting Cytology

Lukas Bubendorf

Insitute of Pathology, University Hospital Basel, Switzerland

Imagine the excitement that the founders of cytology experienced when they first discovered cells under the microscope, and learned how to diagnose disease states just by looking at cellular morphology. Since then, there have been ever growing opportunities for more excitement. While morphology remains the mainstay, new technologies such as immunocytochemistry (ICC), DNA cytometry and fluorescence *in-situ* hybridization (FISH) have widely expanded the utility and diagnostic power of cytology and become part of diagnostic routine in many laboratories. Along with the rapid progress of genomic technologies and personalized medicine, cytopathologists are now being recognized as important players in the field of predictive marker analysis. Prioritizing and preparing cytological material for ICC, FISH and next-generation-sequencing (NGS), data interpretation and integrated reporting have become new tasks requiring special expertise and interdisciplinary collaboration. Liquid biopsies including analysis of circulating tumor cells is another new arena for cytology. Who else than cytologists should be more experienced in analyzing and handling such single cells or small cell groups collected from the blood? Cytopathologists should self-confidently take the new challenges, step into the flashlight and continue the exciting story or cytology.

Disclosure of Interest: Advisor for companies: Pfizer, Roche, Novartis, BMS, MSDResearch support: RocheStock holder of: Novartis, Roche.

IA02-2

[The James W. Reagan Award 2016] Immunocytochemistry: The 3rd Dimension in Cytology

Edneia Tani

Department of Pathology and Cytology, Karolinska University Hospital Solna, Sweden

For many years a cytologic diagnosis was based on a light microscopic analysis of cells stained by MGG, Pap or HE. This procedure allowed a conclusive cytologic diagnosis of many tumors as well as reactive inflammatory lesions. However most poorly differentiated tumors and metastases of unknown primaries could not be definitely classified. In addition lymphomas were impossible to phenotype and sub classify. These difficulties were overcome when immunology was integrated in the cytologic diagnostic work. In the department of Clinical Cytology, Karolinska Hospital, immunologic techniques were adapted to cytologic material beginning in 1986. Over the last 30 years we have used immunocytochemistry in the diagnostic work-up of approximately 30,000 tumors. Smears and cytospin preparations from FNA biopsies or exsudates were proven well suited for immunocytochemistry. Smears are optimal for detection of various nuclear antigens such as hormonal receptors (ER, PgR and AR), Ki67, p63, TTF-1, CDX2, Pax-8, Gata3, WT-1, MUM1, ALK1 and ERG. Cytospin preparations from cell suspension offers on the other hand a perfect material for analysis of cytoplasmic antigens such as cytokeratin subtypes, lymphoid markers, melanoma antigens, various mesenchymal markers as well as MUC antigens.

Cell suspensions of FNA material can also be used for flow cytometry to differentiate reactive lymphadenitis from lymphomas, establish a definitive diagnosis and sub classification of most non-Hodgkin's lymphomas. We have performed flow cytometric analysis over the last 25 years and today about 1000 cases are analyzed every year.

The Karolinska experience of using immunocytochemistry to correctly diagnosis and subtype various tumor has been documented in numerous scientific articles, text book chapters and atlases. The results show that when immunocytochemistry is used to aid cytology most poorly differentiated tumors, lymphomas, mesenchymal tumors and metastases of unknown origin can be diagnosed conclusively. In addition markers for prediction of prognosis as well as therapy response such as hormone receptors, proliferation, EGFR and ALK1 can be accurately identified.

In conclusion, immunocytochemistry is an indispensable adjunct to cytomorphology and its use will distinctly increase the diagnostic accuracy.

Disclosure of Interest: None declared.

IA02-3

[International Cytotechonologist of the Year Award 2015] Clinical Cytology for Everyone – Are We Ready

Celestino Rodrigues Pereira

Department of Pathology Kantonspital Aarau Switzerland, Switzerland

Nil Giris, Tamil Nadu, Southern India: Our Pap Screening Project in Adivasi (indigenous) women is a privately funded program dedicated to assisting the further development of effective, comprehensive Adivasi women's health care in the Gudalur valley and extending the achievements of previous equal endeavors. The main goal of the program is to encourage the health workers to commit to sustainable strategies in the field of prevention of cervical cancer and to bring current systems of preventive strategies into accordance with international strategies and standards.

Data collection/treatment, methodologies/information, and strategies/networking.

Disclosure of Interest: None declared.

IA03-2

[The George L. Wied Award 2016] My Sentimental Journey Through Half a Century of Cytology and Non-Profit Organisations

Louis A. Thienpont

Our Lady's Hospital Aalst, Belgium

A life-time award is an opportunity to look back into the past. In 1957 George Wied initiated and founded the International Academy of Cytology. H.K. Zinser was the first President and G.N. Papancolaou was honorary President. In the sixties, in Belgium and many other countries, screening campaigns for cervical cancer were started up, and cytology departments looked forward to the fascicles with cytologic images and texts G. N. Papanicolaou published at regular intervals. The Belgian Society of Cytology, also founded in 1957, had yearly meetings devoted to GYN cytology and a second meeting every year devoted to cytology of other organs. In 2004, on the occasion of the society meeting in Aalst, the Belgian 'Follow-up Expert Guidelines for Cervical Cytology' were published, making the Bethesda System the official reporting system for all Belgian labs.

Paul Lopez-Cardoso was a pioneer in needle aspiration cytology (FNAC), but it is in the Scandinavian countries that FNAC became common practice in several centres. One of the most prominent centres was, and is, the cytology department of Karolinska Hospital in Stockholm, where I had the opportunity to have a short training. The practice of FNAC of different organs became popular in Our Lady's Hospital Aalst where I was active for most of my professional career. Amongst others, thyroid FNAC was the most popular.

With the introduction of liquid based cytology (LBC) applied in GYN cytology as well as in FNAC, we got access also to immunocytochemistry of aspirated cells as a diagnostic aid.

The Belgian society was founded in 1957 as a factual society with R. Bourg as President and Claude Gompel as secretary. It was incorporated as a non-profit society in 1964. The status of non-profit society (in Belgium vzw/asbl) gives the society a legal status and legal protection as well as a special tax status and the possibility to receive legates.

The International Academy of Cytology was founded as a factual society in Brussels, Belgium in 1957. It became a legal non-profit organisation according to Belgian Law (vzw) on October 12th, 2012 and was officially registered on May 13th 2013 under Nr BE0 534 418 926.

Disclosure of Interest: None declared.

IA03-3

[International Cytotechonologist of the Year Award 2016] Ancillary Cytotechnology: Current Status and Future Perspectives

Hitoshi Itoh

Tokai University Hospital, Japan

We have previously reported that the various technologies such as immunocytochemistry (ICC) and molecular pathology are important tool for cyto-diagnosis. Recently, one of the major advances in cancer treatment is molecular target therapy. The prediction of therapeutic effects depends on the detection of target molecules. Here, we describe multiple technologies which are currently applied to cytology specimens.

(1) Cell Transfer: In clinical cytology, compared with surgical pathology, the unique situation lies in the limited cell amount attached to the slides. This is a method to remove the cells from the glass slides in polymerized plastic plate. Subsequently, the detached plastic is divided into several pieces which could be submitted to multiple methods including ICC and in situ hybridization (ISH) as well as fluorescence in situ hybridization (FISH).

(2) ICC: There have been many reports describing useful immunocytochemical findings in differential diagnosis. Especially, it has been well known that mesothelial cells and mesotheliomas express various markers. We also reported the utility of immunocytochemical markers in making the distinction of mesothelial cells and malignant mesotheliomas from adenocarcinomas on effusion cytology.

(3) ISH: ISH is expected to give inevitable information on various diseases, especially viral infectious diseases. We have successfully applied ISH to detect HPV subtypes on cervical smears. This is important to follow HPV positive cervical dysplasia, especially HPV subtypes are of high risk for carcinogenesis.

(4) FISH, Chromogenic in situ Hybridization (CISH): As the cytology specimen contains whole nuclei, it is considered to be suitable to perform FISH and CISH to detect particular genes and their abnormalities, amplification, deletion and translocation. We have experienced successful application of FISH, CISH and other, techniques on cytology specimens.

(5) EGFR Mutation Analysis: EGFR mutation testing in lung cancer using i-densy® can be successfully applied to archival Pap smears. In our protocol, the slide coverslip is removed and the destaining is performed. DNA extraction are not required in this method.

Future Perspectives: In today's era of personalized medicine, the importance of cytology specimens in diagnostic clinical cytology is expected to expand not only in differential diagnosis but also for therapeutic application. In recent years, 'Next-generation' sequencing technologies have been developed. Several studies have assessed the suitability of cytological specimens, using fresh or fixed smears or cell blocks, for the detection of mutations in several genes. It can be well anticipated that the demand of the molecular technology using cytology specimen will be expanded.

Disclosure of Interest: None declared.

Symposia

SY01-1

Introduction: A Novel Technology and Molecular Cytology

Eiichi Morii

Osaka University, Japan

Cytology deals with the study of cells in terms of structure and function. The change of structure and function is based on molecular events. In this symposium, five invited speakers report recent progresses on novel technologies and cytological studies from the viewpoint of molecules. Tumors derive from a single clone, but consist of heterogenous cell subpopulations whose features and functions are diverse. It has been demonstrated that tumorigenic potential is limited to a small subpopulation known as cancer-initiating cells. Cancer-initiating cells express aldehyde dehydrogenase (ALDH) in a high level. ALDH is known to eliminate various radicals originated from radiation and anti-tumor reagents. Dr. Jun-ichiro Ikeda introduces a new tool against cancers, non-thermal plasma, and discusses its effect on ALDH-expressing cancer initiating cells. To discriminate ductal carcinoma in situ (DCIS) of breast from benign lesion is an important issue in cytopathology. Dr. Yoichiro Yamamoto recently found the morphology of microenvironmental cells was a key point to discriminate DCIS from benign lesion using the computer vision. He introduces this exciting morphometric approach to DCIS. In addition to the computer-associated vision, a new over-1000 nm near infrared imaging fascinates us. This imaging discriminates fat from water, since an over-1000 nm near infrared ray is absorbed in water but not in fat. Professor Yuzuru Ikehara introduces this fascinating imaging in the field of cytopathology and histopathology. Recently, molecular testing is applied to the risk stratification in cytology. Professor N. Paul Ohori introduces FNA cytopathology and the application of molecular testing for risk stratification of thyroid nodules. Lastly, Professor Claire W. Michael, a co-chair, discusses technical differences of two liquid based preparations and discusses how to select which one is best for your laboratory. I hope this symposium will satisfy the audience's curiosity and lead to the progress of cytology.

Disclosure of Interest: None declared.

SY01-2

Anti-Cancer Effect of Non-Thermal Plasma on Cancer Initiating Cells: A New Medical Application for Cancer Therapy

Jun-ichiro Ikeda

Department of Pathology, Osaka University Graduate School of Medicine, Japan

Objectives: Cells with tumorigenic potential are limited to a small population, called cancer-initiating cells (CICs), in many tumors, such as leukemia, breast, brain, and colon cancers. CICs are believed to be cause of cancer recurrence and/or metastasis because they efficiently eliminate anti-tumor chemicals and resist radiotherapy. CICs also have high aldehyde dehydrogenase (ALDH) activity. ALDH could eliminate reactive oxygen species (ROS) or radicals originated from radiation and anti-tumor chemicals. Plasmas, which consist of light, electrons, ions, and radicals, have been used for sterilization of medical equipment, packaging in the food industry, implants, and blood coagulation, mainly using on the thermal effects. Since non-thermal plasma (NTP) device was developed, new medical applications of NTP have been reported for its effect on cancer therapy. As a mechanism of anti-cancer effect of NTP, it is known that electrons, ions and radicals affected the cells to induce ROS, get into cell death by DNA damage, and result in apoptosis. Therefore, we examined the effect of NTP on human uterine endometrioid adenocarcinoma and poorly differentiated human gastric carcinoma cells using ALDH as the target of CICs.

Materials and Methods: NTP treatment was performed in human uterine endometrioid adenocarcinoma cells (HEC-1) and poorly differentiated human gastric carcinoma cells (GCIY). To observe the effect of NTP in more detail, ALDH-high and ALDH-low cells were sorted by flow cytometer, and treated with NTP. The effect of plasma-activated medium (PAM) produced from NTP device on cancer cells was also examined. Additionally, NTP irradiation of tumor xenograft mice and treatment of NTP combined with anti-cancer drug were performed.

Results: When treated with NTP, ALDH-high cells fell into apoptosis in a comparable level to ALDH-low cells as well as PAM treatment. NTP appeared to kill cancer cells more efficiently than anti-cancer drug and combined treatment killed cancer cells more efficiently than only NTP or anti-cancer drug treatment especially at lower doses. In tumor xenograft mouse model, direct irradiation of NTP induced tumor cells apoptosis and decreased tumor cells proliferation and expression of ALDH.

Conclusion: These results suggested that NTP treatment was effective not only on non-CICs but also on CICs. Therefore, NTP might become a new therapeutic approach to CICs.

Disclosure of Interest: None declared.

SY01-3

The Microscopic Scenery through a Computer Vision: New Cytological Morphometric Approach to DCIS

Yoichiro Yamamoto

Department of Pathology, Shinshu University School of Medicine, Japan

The recent progress in the fields of image analysis techniques and artificial intelligence (AI) is remarkable. Supported by the progress of these technologies, big data analysis for morphological features are being developed and put to practical use in diverse fields, such as the face recognition system of a digital camera and the vehicles driving assistance system. We can now use these computer-aided systems even in the pathological field owing to the widespread use of the Whole Slide Imaging System. In this symposium, I will introduce a new morphometric approach for analysis of pathological/cytological images by using AI.

First, all the target cells' nuclei on the slides were measured by image analysis techniques and several dozen morphological features were obtained for each cell. Second, these high dimensional features of each cell were applied to AI. Based on the classification by AI, we calculated the morphological characteristics of cancer cells and microenvironmental cells by each histological classification quantitatively.

Through these results, I will show the strategy by which the current AI recognizes pathological/cytological images and the limit to which the current AI can analyze these image data. Furthermore, I will comment about the possibilities and problems of applying the Deep Learning technique to pathological/cytological images, which has recently drawn many researchers' attention.

Disclosure of Interest: None declared.

SY01-4

Development of a New Imaging Technology to Use Over-1000 nm Near Infrared Wave Length for Histopathology and Cytology

Yuzuru Ikehara[1–3]

[1]Department of Molecular Tumor Pathology, Chiba University, [2]Biotechnology Research Institute for Drug Discovery, National Institute of Advanced Industrial Science and Technology (AIST), [3]Electronics and Photonics Research Institute, AIST, Japan

By the comprehensive analysis with technology of glycoproteomics, we have established biomarkers that might be feasible to use on the early detection of cancers, and on the evaluation of the liver fibrosis progression associated with hepatitis virus infection. Despite of the recent technological advancements to produce such new biomarkers, it is still difficult to develop biomarkers for evaluating progression of diseases such as Alzheimer's disease and age related macular regeneration. Referring back to my experience on researches for new biomarkers development in a last dedicate, it appears that there is one technological limitation. It is that pathologist can't reach directly pathological statuses due to the difficult to see.

Pathological status has been recognized in visible wave length (380–780 nm) to investigate, and the recognition of pathological status is indispensable step to elucidated the underlined mechanisms. Because human retinal cells doesn't response for the light in far-red and ultraviolet, pathological findings to figure out pathological statuses are based on the presence of materials such as hemoglobin and lipids that show absorptions in the ranges of visible wave length. Silicon semiconductors has increased the recognition of pathology in exclusively visible wave length, and promoted the optical sensing to use in the clinical applications on ophthalmology, and computer assisted surgery. Whereas the imaging technologies using silicon sensors are still limited in visible wave length due to the lack of sensitivity for over-1000 nm near infrared wave lengths.

We have developed a camera to detect near infrared wave length with 32000 picture element (VGA) of In0.53GaAs for over-1000 nm and less than 1600 nm near infrared wave lengths. Because InGaAs semiconductors have been widely used for the photon-to-current conversion on optical transmission system, it can be used for sensor camera to visualize the either increase or decrease H_2O in tissue by detecting light absorptions in over-1500 nm wave length. By using this new imaging technology, we succeeded to visualize pancreas and lymph nodes in adipose tissue. Based on such finding, we will discuss the advantages and feasible to use these approaches in clinical practices in this symposium.

Disclosure of Interest: I have (6) research funds from NIKON CO. for developing imaging technology.

SY01-5

FNA Cytopathology and the Application of Molecular Testing for Risk Stratification of Thyroid Nodules

N. Paul Ohori

Department of Pathology, University of Pittsburgh Medical Center – Presbyterian, United States

In the evaluation of thyroid nodules, remarkable progress has been made in the clinical detection of concerning nodules. This has contributed to the increased volume of thyroid cytology fine needle aspiration (FNA) cases over the recent decades. In cytopathology, the implementation of Thyroid Bethesda Classification System has been standardizing the practice of thyroid FNA cytology. However, conventional cytomorphologic analysis alone is reaching its maximum potential and additional techniques are needed to improve diagnostic sensitivity and specificity of the FNA procedure and to decrease the number of diagnostic surgical resections. Among the recent advancements, the application of molecular techniques in conjunction with routine cytologic examination holds promise, especially with regard to the indeterminate thyroid cytopathology diagnoses. For example, the atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm/suspicious for follicular neoplasm (FN/SFN) diagnoses carry concern for malignant outcome, yet the majority of corresponding resected cases show benign outcome.

Development of molecular tests in this area has been highly kinetic with a variety of modalities available for testing. In general, three approaches are used currently: 1) testing individual markers by immunohistochemistry or molecular methods, 2) panel approach for mutations associated with thyroid cancer (e.g. next-generation sequencing), and 3) panel approach by identifying a profile of markers associated with benignity (e.g. *Affirma* gene expression classifier 'rule-out' test). Overall, molecular tests have shown that ancillary information is helpful in improving nodule characterization and risk stratification. We will discuss the advantages and shortcomings of these approaches.

Disclosure of Interest: None declared.

SY01-6

ThinPrep versus SurePath: Which One Is Best for Your Laboratory?

Claire W. Michael

Case Western Reserve University/University Hospitals Case Medical Center, United States

Introduction: Liquid based preparations (LBP) have been introduced as automated alternative processing techniques to direct smears and cytospins. Two such methodologies are currently approved for cytopathology diagnosis.

Methods and Results: ThinPrep is based on filtration of cells through a pneumonic vacuum chamber while SurePath is based on cellular enrichment and sedimentation. Material is collected in CytoLyt a methanol based preservative for ThinPrep and in CytoRich an ethanol based preservative for Sure Path. LBP are generally easier to examine because of the loss of obscuring elements, thin layer with even distribution of cells, better cellular preservation and smaller area to screen. The steps involved and the space requirement vary between these two techniques. While both result in a ThinLayer, ThinPrep produces a more flat Prep while SurePath produces a more 3-dimentional Prep.

Conclusion: While both techniques are acceptable and superior to traditional techniques, Although SurePath is easier to review for the beginner, both produce their own unique cytological artifacts that pathologists need to be familiar with. This presentation will discuss the different methodologies, required space and expense involved, technical problems and artifacts for the two LBP.

Disclosure of Interest: None declared.

SY02-1

Pre Analytical Issues with Breast FNAB

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This talk will address the current role of fine needle aspiration biopsy (FNAB) in assessing breast lesions, the techniques required to optimize yield and the value of this biopsy technique.

The indications and contraindications for FNAB of both palpable and radiologically-detected breast lesions will be discussed with reference to differing medical resources and clinical settings including remote/rural practice, general practice, specialist breast clinics and screening programs.

The current widespread use of the core biopsy technique, predominantly performed under radiological guidance, has changed practice in many parts of the world and in settings such as radiologically-detected calcifications offers greater sensitivity than FNAB. However, the two techniques should be complementary as there are disadvantages of the core biopsy procedure in certain clinical situations and FNAB of breast lesions remains a simple, cost effective and highly sensitive procedure if performed by well-trained clinicians and interpreted by cytopathologists with experience in breast FNA. The added psychological benefit of providing an immediate result to the patient should not be underestimated.

In the developed world, where cost constraints are always of consideration, FNAB plays a valuable role and its diagnostic accuracy in palpable breast lumps has been reported to be up to 99%, when used in conjunction with imaging and clinical features (triple test). The overall sensitivity of FNAB varies in different studies but is generally between 70–90%.

In resource – limited settings the cost of core needle biopsy is relatively greater, implying a greater potential value of the FNAB technique.

Ideally, rapid on site evaluation (ROSE) would be utilized to confirm diagnostic material, but in many practices this is not a viable option and it is important that clinicians (including surgeons, radiologists and general practitioners) are trained adequately in smear preparation to minimize the problems associated with inadequate or insufficient material. As there is no universally accepted definition of adequacy in breast FNAB, this is addressed in relation to a range of specific clinical settings.

The requirement for evidence based structured reporting is now established in many fields of pathology and attention to pre-analytical issues will ensure a reproducible cytopathology report in breast FNAB and aid in maximizing the usefulness of this technique and optimizing communication with the clinicians and patient.

Disclosure of Interest: None declared.

SY02-2

Proliferative Breast Lesions: Cytological Diagnostic Criteria of Benign Lesions and the Concept of 'Atypical Lesions'

Andrew S. Field

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Breast FNAB has a long and successful tradition and is used internationally but its usage varies greatly from country to country, city to city and FNAB practice to practice. It has been and continues as a cost effective and accurate diagnostic tool in the developed world. In the developing world where there are limited medical resources including diagnostic radiology for mammography and breast ultrasound and surgical pathology laboratories for breast core and excision specimens, the potential for FNAB to radically improve the diagnosis and management of breast cancer is huge. But FNAB of breast lesions has a number of specific problem areas in diagnosing benign proliferative lesions. Benign proliferative lesions such as fibroadenomas and intraduct papillomas show a huge range of features both in surgical pathology and cytopathology. Proliferative lesions such as fibroadenomas and intraduct papillomas can produce high cellularity, which is usually associated in FNAB work with malignant lesions. Diagnostic criteria for proliferative lesions can overlap with malignant lesions in the breast, for example, the large epithelial tissue fragment pattern can be seen in epithelial hyperplasia, fibroadenomas, intraduct carcinomas and invasive carcinomas, and stromal cellularity in fibroadenomas overlaps with phyllodes tumors. The full FNAB diagnostic criteria for proliferative lesions have to be assessed in each case, and these will be briefly presented and discussed. The aim of the IAC initiative to produce a standardized approach to FNAB breast includes establishing diagnostic categories with diagnostic criteria, that can be used in reporting and the establishment of management recommendations.

Disclosure of Interest: None declared.

SY02-3

Role of Ancillary Tests in Reporting Breast Cytology and a Classification System

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Pathologists' role is imperative in the cancer management of all systems, however, nowhere is this more evident than in the place of breast cancer patients thorough the significant advances in molecular biology, thus the tumor heterogeneity of breast carcinoma. Biomarkers in routine of breast pathology might be sum up in three parts as 'prognostic markers, 'predictive markers' and 'diagnostic markers'. Prognostic biomarkers are independently forecast of clinical outcome (i.e., risk of recurrences, mortality). On the contrary, predictive biomarkers determine response of the given therapy. Diagnostic biomarkers are mainly decide the biologic nature of the breast lesions whether or not benign versus malignant and the source of metastasis.

There are some markers used to demonstrate the presence of basal/myoepithelial cells, which help in the differential diagnosis between benign and malignant lesions on cytology. CK5, CK14, CK17, SMA, Calponin, P63 are markers described in the literature, being p63 very useful in cytology because is a nuclear marker. Also E-Cadherin, CK34B12 and CK8 are helpful to distinguish ductal carcinoma from lobular carcinoma. These markers are particularly useful to reduce the intrinsic limitations of FNA owing to small sample size and the lack of the reliable histopathologic architecture. Using these markers in cytology routine practice will be prevent false negative or false positive results and help to properly categorize malignant, benign and low grade/borderline breast lesions.

Beside the diagnostic markers used in primary lesions, also there is others that allow to identify breast carcinoma origin in metastatic lesions such as GATA-3, GCDFP-15 and hormone receptors. Sampling by fine needle aspiration is extremely helpful for metastatic sites of cancer patients, also sampling of these sites is not only important for the routine practice but also enormously important for the clinical trials to understand the discordance of mutations between primary breast cancer and its metastatic lesions that will be enlightened the clonal heterogeneity and the metastasis biology to set up the better categorization and targeted therapies to improve survival and the quality of life. Various numbers of potentially predictive factors have been also identified but only three biomarkers are used in current clinical routine; ER, PR and HER2. These factors can be detected mostly by immunohistochemistry (IHC), also fluorescent in situ hybridization (FISH) and gene assays can be used in some conditions based on the guidelines. The assessment of these three biomarkers is mandatory since their results have a crucial impact on the management.

Disclosure of Interest: None declared.

Symposium 3

Pathology and Cytology of Ovarian Tumor

Chairs: *Tsunehisa Kaku* (Department of Health Sciences, Kyushu University, Japan)
Masaharu Fukunaga (Department of Pathology, Shin-Yurigaoka General Hospital/Jikei University Daisan Hospital, Japan)

The cytological examination of ovarian tumor involves three types of specimens; peritoneal fluids obtained by peritoneal ascetic fluid or peritoneal washing fluid, intraoperative touch smear of tumor, and fine-needle aspirates of ovarian cysts and peritoneal fluids. The purposes of cytology for ovarian tumors are distinguishing non-neoplastic cyst from neoplastic tumor, detecting disseminated ovarian cancers and disease suspected pelvic recurrence of ovarian cancers, ascitic or pleural fluid of malignant cells and peritoneal washings at the operation to detect peritoneal lesions. In this symposium 'cytology and pathology of ovarian tumor', cytological features of epithelial tumors (serous tumors, mucinous

tumors, endometrioid tumors, clear cell tumors, Brenner tumors and transitional cell tumors), sex cord-stromal tumors, germ cell tumors, and others are presented in relationship with pathologic features of each tumors.

SY03-1

Ovarian Epithelial Tumors: An Overview

Steven G. Silverberg

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Ovarian tumor specimens received by the pathologist for examination include: (1) fine needle aspirations (FNA); (2) core needle or other biopsies; (3) resections; and (4) tissues peripheral to the presumed ovarian tumor (metastases, ascitic fluids, etc.). Questions to be answered comprise: (1) is it truly ovarian? (rule out parovarian/tubal/metastatic); (2) is it neoplastic? (rule out massive edema, stromal hyperplasias, inflammation, endometriosis, etc.); (3) if neoplastic, in which category? (epithelial or other); (4) if epithelial, of what histopathologic type?; (5) benign, borderline, or carcinoma?; (6) if appropriate, what is the tumor grade?, and (7) other features (pathologic stage, tubal STIC, endometriosis, etc.). Relatively few large series of ovarian tumors analyzed by cytopathology have been reported, but in general terms the results for issues 2, 3 and 5 are the best (with borderline tumors often a problem), with 4 and 6 less satisfactory, and 1 and 7 usually impossible without large histopathologic specimens.

In the new (2014) World Health Organization (WHO) classification of ovarian tumors, major differences from the previous classification (2003) are (1) seromucinous tumors have become a separate category, together with serous, mucinous, endometrioid, clear cell, and Brenner tumors (as a result, the term 'intestinal' is no longer used for mucinous tumors, since they are all of that type); (2) transitional cell carcinoma has disappeared (now considered mainly a variant of high grade serous carcinoma); (3) mixed epithelial tumors have also disappeared (a rather convoluted explanation is provided, and will be discussed in this full presentation); (4) the grading system of well, moderately, and poorly differentiated (grades 1/2/3) serous carcinoma has been replaced by two separate categories designated 'low-grade' and 'high-grade' serous carcinoma, and (5) the terms 'non-invasive' and 'invasive implants' for peritoneal lesions associated with serous borderline tumors have also been replaced by 'serous borderline tumor' and 'low-grade serous carcinoma', respectively (high-grade serous carcinoma is strangely absent here, although it certainly exists).

These classification changes will be discussed in more detail as a preamble to the full discussions of the individual entities by my colleagues in this program.

Disclosure of Interest: None declared.

SY03-2

Cytology of the Ovarian Serous Tumors

Tetsuro Nagasaka

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Objectives: Serous tumors are the most common ovarian neoplasms that include benign cystadenomas, borderline tumors and adenocarcinomas. Adenocarcinomas are divided to low grade and high grade in new WHO classification. This presentation will display the characteristic cytological findings of the serous tumors.

Materials and Methods: Several collected cases (peritoneal washes and stump preparations) of the related hospitals were reviewed.

Results:

Serous Cystadenoma: The cells form tightly cohesive aggregates and simple papillae of small cells, which may be associated psammoma bodies. Few or no single epithelial cells are present. Cilia are characteristic.

Serous Borderline Tumor: Ovarian serous borderline tumors are noninvasive histologically. Cytologically, these tumors are characterized by the presence of cohesive, often branching, papillary structures with smooth borders. Low-grade nuclear atypia, consisting of minor variation in size, irregular membrane, coarse chromatin, and occasional prominent nucleoli, and single cells may be seen, but mitosis are rare or absent. Psammoma bodies may be present.

Serous Adenocarcinoma: Compare with borderline tumors, serous adenocarcinoma is characterized by a predominance of smaller cohesive clusters and papillae, with more irregular borders and increase in single cells. These cells larger and more atypical, and the cytoplasm is abundant and usually vacuolated. Mitosis may be frequent. Psammoma bodies can be seen.

Differential diagnosis: There is morphologic spectrum from reactive mesothelial cells to endosalpingiosis to borderline serous tumors to serous adenocarcinoma. The cells of these conditions can appear quite similar to one another with only subtle differences. The differential diagnosis of reactive mesothelial cells with low grade adenocarcinoma is based on finding orderly flat sheets of cells with smooth nuclear membrane and bland chromatin vs. disorderly, three-dimensional clusters of cells with irregular nuclear membranes and abnormal chromatin.

Conclusions: Cytological examinations are useful for differential diagnosis especially in peritoneal washes. But it is impossible to differentiate between serous borderline tumor and low grade serous adenocarcinoma based on the cytology alone. Histopathological examination is necessary to confirm the diagnosis.

Disclosure of Interest: None declared.

SY03-3

Pathology and Cytology of Ovarian Endometrioid Tumor and Clear Cell Tumor

Suzuko Moritani

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According to the latest WHO classification of female reproductive organs (2014), both endometrioid and clear cell tumors have benign, borderline, and malignant counterpart, as well as other epithelial ovarian tumors do. However, benign and borderline counterpart of clear cell tumor are extremely rare. Similarly, borderline counterpart of endometrioid tumor is relatively rare. In this symposium, we review the cytology and pathology of selected lesions from those categories which we frequently encounter in our dairy practice.

Endometriotic cyst, the most common benign endometrioid tumor, is characterized by endometrial epithelium and stroma admixed with fresh and old hemorrhage. Touch smear preparation of the cyst wall shows clusters of epithelial cells with small round nuclei with hemosiderin-laden macrophages in the background. There are occasionally clusters of mononuclear cells with indistinct cytoplasm derived from stromal component. Metaplastic epithelial cells with abundant cytoplasm stained with light green often have enlarged nuclei and such cells should not be regarded as malignancy.

Endometrioid carcinoma is associated with endometriosis in up to 42% of the cases. Neoplastic epithelial cells resemble those of endometriotic cyst when the tumor is well differentiated. Irregular branching and papillary clusters reflect architectural abnormality and are clues to consider lesions of borderline or more. Squamous differentiation, if present, could be a hallmark of endometrioid tumor. In addition to squamous differentiation, endometrioid carcinoma occasionally show a variety of cytological appearances, such as mucinous, eosinophilic, ciliated, and spindle cell changes. It is rare to see neoplastic cells of endometrioid carcinoma in ascites comparing to serous carcinoma. If a small number of neoplastic cells of low-grade endometrioid carcinoma exist in ascites, it would be difficult or even impossible to differentiate these cells from those of endometriosis, endosalpingiosis, and normal tubular cells.

Like endometrioid carcinoma, clear cell carcinoma is closely related to endometriosis. Histologically the tumor consists of tubulopapillary or solid growth of clear neoplastic cells. Tubules lined by hobnail cells and small papillae with central hyalinized stromal core are also characteristic. Touch smear preparation of the tumor shows sheets or three dimensional clusters of neoplastic cells with clear translucent cytoplasm. Reflecting small papillae, there are spherical hyalinized material surrounded by neoplastic cells. The hyalinized material shows metachromasia in air-dried smear with Giemsa stain. Tumor cells in peritoneal fluid have the same appearance.

Disclosure of Interest: None declared.

SY03-4

Cytology and Pathology of Ovarian Mucinous Tumor

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Mucinous Cystadenoma/Adenofibroma: A benign, cystic tumor is lined by mucinous gastro-intestinal-type epithelium or rarely having prominent fibrous stroma (adenofibroma). The typical tumors are composed of multiple cysts and glands lined by simple, nonstratified mucinous epithelium resembling gastric or intestinal epithelium containing goblet cells and neuroendocrine cells, or Paneth cells. Focal papillae are often seen.

Cytologically, the mucinous cystadenomas show a few isolated columnar cells or a few sheets of columnar mucin producing cells with abundant mucin in the background. Mucinous cells often arrange one or two layers of mucin. Nuclei are round or with fine chromatin pattern, and mitotic figures are not seen.

Mucinous Borderline Tumor: The cysts are lined by gastrointestinal type epithelium in the form of gastric pyloric-type epithelium, goblet cells, neuroendocrine cells and Paneth cells. The epithelium exhibits varying degree of stratification, tufting and villous or slender papillae. The cells show mild to moderate nuclear enlargement, hyperchromasia and pseudostratification, but high-grade nuclear features are not seen. The mitotic index varies from slight to brisk.

Pseudomyxoma ovarii is often present. Grossly mural nodular can be associated with mucinous borderline tumor.

Cytologically, borderline mucinous tumors are difficult to differentiate from malignant counterpart as invasion cannot be documented on cytology. Borderline mucinous tumors are composed of cohesive sheets of bland cells and peripheral palisading by mucin-filled cells. They usually present as highly cellular smears with nuclear features ranging from bland to highly atypical.

Mucinous Carcinoma: There is often a continuum of architectural and cytological atypia that includes benign, borderline and frankly carcinomatous areas. Invasive carcinoma is characterized by two different patterns of invasion. The confluent glandular and/or expansile invasive patters are recognized by marked glandular crowding with little intervening stroma, and creating a labyrinthine appearance. A cribriform pattern is often seen. Mitotic activity is often quite high and abnormal mitotic figures are frequently present. Tumor heterogeneity is common in mucinous neoplasms, so that areas of benign and borderline histology are often coexist in the tumor. Areas of anaplastic carcinoma are sometimes present, usually focally, and forming so-called mural nodules.

Cytologically, mucinous carcinomas show pleomorphic and irregular nuclei, prominent nucleolei and vacuolated cytoplasm with mucin. Overlapping of malignant cells and glandular structures are also noted. Mitotic figures are often seen. The necrotic debris and mucin are seen in the background. The identification of metastatic tumors is extremely difficult in cytology unless relevant clinical history and investigation reports are supplied.

Disclosure of Interest: None declared.

SY03-5

Pathology and Cytology of Ovarian Sex-Cord Tumor and Other Ovarian Lesions

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Imprint cytology plays a great role in intraoperative pathologic consultation. Here, classic cases of ovarian sex-cord tumors and other challenging or pitfall ovarian lesions, including metastatic tumors, are presented and their cytologic and histologic features are described.

As majorities of sex-cord tumors are benign or boderline malignant, they very rarely yield ascitis materials and imprint smear or FNA is most frequent procedure.

Smears from granuloma cell tumors are cellular and contains clumps as well as individual cells. Microfollicular structures (Call-Exner bodies) are helpful diagnostic features if present. Individual cells have uniform small round or oval hyperchromatic nuclei, small nucleoli and variable amounts of cytoplasm. Nuclear coffee-bean like grooves are relatively characteristic findings. This tumor can be distinguished from Brenner tumors, which show nuclear grooves and fibroblasts-like spindle cells but lack the microfollicular structures. Juvenile granulosa cell tumors differs cytologically from adult counterpart by the lack of prominent grooved nuclei, absence of Call-Exner bodies and the presence of enlarged nuclei.

In Sertoli cell tumors, tumor cells show uniform oval nuclei with prominent nucleoli and irregularly distributed chromatin. They often show tubular structures. It is difficult to differentiate Sertoli cell tumor from granular cell tumor in cytological material. In some cases, luteinized cells with more abundant, finely vacuolated cytoplasm may be present. Poorly differentiated Sertoli cell tumors are characterized by the presence of atypical cells and sarcomatous pleomorphic cells; however, it is very difficult to differ from other poorly differentiated or pleomorphic tumors.

Fibromas or thecoma are composed of spindle or oval cells, either isolated or in bundles, with scant cytoplasm and elongated nuclei. Cells of thecoma have oval or round nuclei and luteinized cytoplasm. Luteinized cells are often seen in sex-cord tumors, but they may be observed also in stromal hyperplasia, hyperthecosis or metastatic carcinoma and induce clinical estrogenic effects.

Cytological diagnosis is useful in the context of full knowledge of the clinical and radiographic findings.

Disclosure of Interest: None declared.

SY03-6

Peritoneal Fluid Cytology and Pathology of Ovarian Germ Cell Tumor

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Objectives: Ovarian germ cell tumors rarely exfoliate tumor cells in peritoneal fluid, and the cytological features are not well described. We present and discuss peritoneal fluid cytology of the histologically confirmed germ cell tumors including dysgerminoma, yolk sac tumor and pseudomyxoma peritonei derived from mature cystic teratoma.

Results:

Dysgerminoma: On ascitic fluid smears, the tumor cells were seen singly or in pairs. Rarely they were in closely packed clusters of a dozen or more cells. The tumor cells were predominantly uniform with large, oval or round fairly well preserved cytoplasm. The nuclei were large, oval or round with prominent nucleolei. The nuclear chromatin was fine and evenly distributed without condensation, giving a clear appearance to the nucleus. The nuclei were located centrally in the cytoplasm. Occasionally, a pale brown coarsely granular substance was seen in the cytoplasm. Histologically, ovarian tumor cells were large and round and resembled primordial germ cells.

Yolk sac tumor: The smears of ascetic fluid showed a clean background, with a few scattered mesothelial cells and poorly preserved atypical cells loosely arranged in irregular or papillary groups. The cells had ill-defined and microvacuolated cytoplasms, with an elevated nuclear-cytoplasmic ratio and prominent nucleolei. PAS-positive, diastase-resistant intracytoplasmic and extracellular hyaline globules, which stained intensely for AFP by immunocytochemistry, were observed. Histologically ovarian tumor showed network of spaces lined by small cuboidal cells, which were positive for AFP.

Pseudomyxoma peritonei caused by mature teratoma: The fluid was quite viscous, whitish and PAS-positive. The smear disclosed the characteristic mucus-producing columnar cells exhibiting minimal cytological atypia. Large sheets of fairly uniform columnar or cuboidal cells were floating in thick mucus. Fibroblastic mesenchymal cells were also observed. Histology of right ovarian tumor is mature cystic teratoma, composed of intestine, skin appendages, respiratory epithelium, bone and cartilage. No abnormal findings were observed in the appendix.

Discussion: The cytological features of dysgerminoma and yolk sac tumor in peritoneal fluid are sufficiently characteristic to make specific diagnosis possibly in technically high quality smears. Cytological findings of pseudomyxoma peritonei caused by ovarian teratoma might differentiate from those caused by appendiceal tumors.

Disclosure of Interest: None declared.

SY04-2

Study on the Morphology and Reproducibility of the Diagnosis of Endometrial Lesions Utilizing Liquid-Based Cytology

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Objectives: Until 2010 the reporting of endometrial cytologic was according to the 1994 classification scheme of the World Health Organization. The disadvantage of this scheme was the difficulty in the communication between the cytopathologists and gynecologists. From 2010 up to 2012 a new classification scheme of LBC samples, in cooperation with gynecologists, was developed. Aim of this study is to determine the diagnostic cytomorphologic criteria and to evaluate the reproducibility and usefulness of the new classification scheme.

Materials and Methods: The new classification scheme has the following categories: inadequate, without evidence of malignancy, atypical endometrial cells with low probability for malignancy (ACE-L), atypical endometrial cells with high probability for malignancy (ACE-H), atypical endometrial cells of undetermined significance (ACE-US) and malignant. Moreover the risk of malignancy was calculated. The study involved 1275 cases (excluding inadequate) that had histological confirmation after hysterectomy. After establishment of the criteria, five cytopathologists examined a part of the cases in order to determine the intraobserver variability.

Results: The quality indices of the new classification scheme on the basis of the 1275 cases was Sensitivity = 93.70%, Specificity = 98.88%, PPV = 97.28%, NPV = 97.36% and Overall Accuracy = 97.33%. The Kendall's coefficient of concordance for the ordinal response of the five raters was W = 81.47% ($p < 0.0001$), indicating excellent concordance among the raters. However the greatest disagreement was fond on ACE-US cases (kappa = 0.11, $p = 0.0065$). The risk of malignancy for each diagnostic category was: for the inadequate cases lower than 20%, for the without evidence of malignancy cases about 1%, for ACE-L about 10%, for ACE-US about 70% (6 cases), for ACE-H about 85% and for the malignant cases almost 100%. The ROC curve for the comparison of the cytological result using as golden standard the histological outcome had AUC about 96%.

Conclusions: Liquid-based cytology allows for standardized and reproducible endometrial preparations, which in turn allows the application of common diagnostic criteria among cytopathologists. Furthermore, LBC in combination with endometrial sampling could be a useful tool for the outpatient diagnosis of endometrial lesions, which could reduce unnecessary curettage. The new six tier classification scheme can be easily applied in the everyday routine of cytopathology laboratories, additionally is a tool that facilitates communication between cytopathologists and gynecologists.

Disclosure of Interest: None declared.

SY04-3

Morphological Features of Endometrial Liquid-Based Cytology (LBC) Compared to Conventional Method and Its Performance Values

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Objectives: Cytological findings of liquid-based cytology (LBC) are different from those of conventional method. The purpose of the study was to compare the morphological features of both methods using endometrial cytology and to clarify the performance values of endometrial LBC.

Materials and Methods: Split endometrial cytology specimens prepared in LBC and conventional method, including cases of endometrial endometrioid adenocarcinoma, Grade1 (G1) and Grade2 (G2) were used. Eight findings (the number of cluster layer, raveling from cluster, the number of cell cluster per unit area, cell number of cluster, major axis of cluster, nuclear size, nuclear roundness and nucleolar size) were compared between the two methods.

Results: The number of layer in G1 was more significantly in LBC than in conventional method. Raveling from cluster in G1 and G2 was the same proportion in both methods. The number of cell cluster per unit area in G1 and G2 was more in LBC. Cell number of cluster, major axis of cluster, nuclear size, nuclear roundness in G1 and G2 showed a significantly low value in LBC. Nucleolar area in G1 and G2 was higher in LBC.

Conclusion: It was revealed that 1) Structural atypia was preserved, 2) Tumor cells were efficiently collected, 3) Nucleolus was prominent in LBC, compared to the conventional method. Therefore, it was indicated that LBC method may be a useful method by understanding the characteristic cell findings in LBC.

Disclosure of Interest: None declared.

SY04-4

Expression of PTEN in Endometrial LBC

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Objectives: Endometrial cytology is an alternative to biopsy in endometrial cancer diagnosis. The introduction of liquid-based cytology (LBC) and endoflower dispositive in routine practice

gives the possibility to examine endometrial cells by cytological diagnosis and also release the opportunity to apply ancillary techniques to study molecular alterations. In particular, PTEN evaluation could be useful in endometrial carcinomas for selecting patients for target therapies.

Material and Methods: We studied 51 endometrial samples collected using the Endogyn device and 71 obtained with the Endoflower dispositive device, and processed using liquid-based cytology. Most of the cases were matched with a corresponding histological biopsy. The overall accuracy of Endoflower was 100%. Immunohistochemistry (IHC) and immunocytochemistry (ICC) for PTEN were performed using monoclonal antibody 6H2.1 from DAKO.

Results: We found PTEN-null glands in 4 cases in IHC and in ICC. Over 10 carcinomas analyzed on cytology, 1 case showed PTEN-null glands. All the normal endometrium control cases were positive in cytology and histology.

Conclusion: Our results suggest that it is possible to use endometrial LBC samples collected with an office-based painless cytological dispositive to diagnose endometrial diseases. The evaluation of PTEN loss by ICC is possible with the application of standard techniques, with concordant IHC results.

Disclosure of Interest: None declared.

SY04-5

Classification of Endometrial Lesions by Nuclear Morphometry Features Extracted from LBC Samples

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Objectives: Objective of this study is to investigate the potential of classification and regression trees (CARTs) in discriminating endometrial lesions by nuclear morphometry features into lesions with benign biological behavior or malignant biological behavior based on objective nuclear morphometric criteria.

Materials and Methods: The study was performed on 222 histologicaly confirmed liquid based cytological smears taken by direct sampling of the endometrial cavity with the EndoGyn® Sampler. Specifically: 117 benign cases, 62 malignant cases and 43 hyperplasias with or without atypia. About 100 nuclei were measured from each case using an image analysis system. The nuclei from 50% of the cases (the training set) were used to construct a CART model. The nuclei from the remaining 50% of the cases (test set) were used to evaluate the stability and performance of the CART on unknown data. Classification of individual cellular nuclei into two categories according to the need for D&C. The output of the CART for nuclei classification was exploited to construct, two different case classifiers.

Results: The CART model had overall accuracy for classifying endometrial nuclei equal to 85%, while specificity was 90.68% and sensitivity was 72.05%. Both methods for cases classification had statistically significant similar performance: overall accuracy: 94–95%, specificity: 95% and sensitivity: 91–94%.

Conclusions: The clinical application of the CART model is towards the discrimination of endometrial lesions as an additional tool to the cytological diagnosis. The clinical value is in the triage of cases requiring immediate referral to D&C from those that could be treated by pharmaceutical therapy or monitoring through follow up. The proposed study highlighted interesting diagnostic features of endometrial nuclear morphology. The proposed method can be a useful tool for the everyday practice of the cytological laboratory.

Disclosure of Interest: None declared.

SY04-7

Assessment of Endometrial Pathology: Diagnostic Reproducibility in LBC Samples

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Introduction: Endometrial cytology is the common method for diagnosis of endometrial disease in Japan. Up to now, the diagnosis made by using conventional preparation method, occasionally there is that the discrepancy occurs between cytological and histological findings.

Method: We studied endometrial samples which included the cases of normal endometrium, hormonal dysfunction status (EGBD etc.), endometrial hyperplasia, endometrioid adenocarcinoma (G1 and G2), mucinous carcinoma, endometrial serous carcinoma and clear cell carcinoma, processed using BD SurePath-LBC preparation (SP) method. The comparison studies between cytological findings in LBC preparation and findings in histological preparations were made. Especially in the cases of those endometrial carcinomas, immunohistochemial and immunocytochemical studies were carried out together in a same case, using a variety of antibodies (ER, p53, Ki-67, beta-catenin, IMP3, Napsin A etc.).

Result: We found that endometrial epithelial cell clusters in SP were well retained and the structures of these cell clusters corresponded to histological features in each endometrial disease. Also concerning to the result of immnocytochemical staining status of each antibodies in SP, it showed almost the same status of immnohistological stained preparations in sensitivity and intensity.

Conclusion: LBC method for endometrial samples shows good reproducibility for the diagnosis of endometrial lesions, morphologically. It is possible to examine with immnocytochemical method routinely, and it seems to provide the reliability and accuracy in diagnosis of endometrial disease. Finally LBC method for endometrial samples will become effective method as microbiopsy.

I declear that I have no conflict of interest.

Disclosure of Interest: None declared.

SY04-8

Proposal of the Descriptive Reporting Format for Endometrial Cytology Devised by the JSCC Study Group

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The new standardized descriptive reporting format for endometrial cytology is mandatory to make more reliable endometrial cytology. In 2008, a clinical study for evaluating sensitivity and specificity of endometrial cytology obtained by intrauterine sample in Japan was carried in the Japanese Society of clinical cytology (JSCC) group. For this clinical study, we developed a descriptive reporting format. In this reporting format, cytological result were classified as 'negative for malignancy', 'atypical endometrial cells (ATEC)', 'endometrial hyperplasia', 'atypical endometrial hyperplasia', and 'malignant tumor'. ATEC was subclassified as 'ATEC, of undetermined significance' 'ATEC-US) and 'ATEC, cannot exclude atypical endometrial hyperplasia or more' (ATEC-A). While a more detailed cytological diagnosis can be selected for cases of 'negative for malignancy', 'endometrial hyperplasia', 'atypical endometrial hyperplasia', or 'malignant tumor' with endometrial cytology, for cases evaluated as ATEC, either an ATEC-US or ATEC-A has to be selected without exception. When the cytological result is 'negative for malignancy', subsequent endometrial histological evaluation is not necessarily required. In case of ATEC-US, endometrial biopsy or repeat endometrial cytological assessment after 2 or 3 months is required. When the cytological result is other than 'negative for malignancy' or ATEC-US, endometrial biopsy or curettage is required to confirm the endometrial diagnosis. In 2015, this descriptive reporting format for endometrial cytology was published in the JSCC atlas and guidelines for cytopathological diagnosis (Vol.1). We believe that the descriptive reporting format for endometrial cytology devised by the JSCC study group could be the basis of universal reporting format, which is expected to be improved based on the reliable evidences.

Disclosure of Interest: None declared.

SY04-9

The Evaluation of Endometrial Cytology by Diagnostic Flow Chart That Provide Simple, Standardized, and Reliable Diagnostic Criteria

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Introduction: Recently the Osaki Study Group (OSG) proposed new cytological diagnostic criteria that can be used with the standardised and automated sample processing methodology of BD SurePath (SP)-liquid-based cytological (LBC) system. This new cytological diagnostic criteria has been called: 'diagnostic flowchart of the OSG method' (so called OSG method), and is a simple diagnostic method consisting of three steps. The first step evaluates whether the morphology of the cell clumps is regular or irregular and whether nuclear overlapping amounts to more or less than three layers. Next, a cytomorphological evaluation of endometrial samples considering various physiological or physiopathological situations of the endometrial mucosa is performed and in the third step the final diagnosis is finally made.

The OSG method terminology for final diagnosis consists of six categories:(1) normal endometrium (cycling, atrophic endometrium; NE), (2) Endometrial glandular and stromal breakdown (EGBD), (3) atypical endometrial cells, cannot exclude atypical endometrial hyperplasia or more (ATEC-A), (4) adenocarcinoma including atypical endometrial hyperplasia or malignant tumour (Malignancy), (5) endometrial hyperplasia without atypia (EH), (6) Atypical endometrial cells of undetermined significance (ATEC-US).

Method and Results: The sensitivity and specificity of SP-LBC preparation according to the OSG method was evaluated in 122 endometrial samples, the diagnostic sensitivity (96.4%) and specificity (100%) of the OSG method were both very high. Moreover, the reproducibility of interobserver and intraobserver agreement by 3 cytopathologists that were reviewed for first and after second round at 3-month intervals was evaluated in 244 endometrial samplings.

The interobserver agreement of NE classes improved progressively from 'good to fair' to 'excellent', with values increasing from 0.70 to 0.81. Both of EGBD and Malignancy classes improved progressively from 'good to fair' to 'excellent', with values increasing from 0.62–0.63 to 0.84–0.95 respectively. The overall intraobserver agreement between the first and the second rounds was 'good to fair' to 'excellent', with values changing from 0.79 to 0.85. All kappa improvements were significant ($p < 0.0001$).

Conclusion: It considered that use of the OSG method as the new diagnostic criteria by SP-LBC preparation may be a valid method to improve precision of endometrial cytology.

Disclosure of Interest: None declared.

SY05-1

The Japan Thyroid Association Reporting System of Thyroid Cytology – Resection Rates and Risks of Malignancy of Indeterminate Nodules

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Objectives: The Japan Thyroid Association (JTA) published a new reporting system for thyroid cytology, with three points differing from existing internationally-accepted systems. First is the sub-classification of the so-called indeterminate category, which is divided into 'follicular neoplasm (FA/FTC lineage)' and 'others (PTC lineage)'. Second is the risk-classification of follicular neoplasm (FN) into 'favor benign', 'borderline' and 'favor malignant'. Third is the use of self-explanatory terms for histological type and probability of malignancy. The JTA system is designed for further risk stratification of patients with indeterminate cytology, which is recommended in the JTA clinical guidelines. Performance of the JTA system is characterized by low resection rates and high proportions of malignancy in patients with indeterminate cytology. This is due to further triage of patients using ultrasound and other clinical tests traditionally carried out in Japan.

Results: The resection rate and risk of malignancy of favor benign FN were 53.5% and 10.7%, those of borderline FN were 87.5% and 33.3%, and those of favor malignant FN were 100% and 45.5%, respectively, as reported by Satoh et al from the Yamashita Clinic. Kameyama et al developed a scoring system to sub-classify FN using 3 parameters, cellularity, nuclear overlap, and nuclear atypia, and demonstrated that the risk of malignancy of favor benign FN was 11.5%, that of borderline FN was 53.8%, and that of favor malignant FN was 81.8%. Zhu et al from China reported their resection rate of the Thy3 category was 41.6% and the risk of malignancy was 51.9%, and the risk of malignancy of subcategory Thy3-PTC was 71.4%, that of Thy3-FN was 46%, that of Thy3-HN was 42.9%, and that of Thy3-FL was 27.3%. Thus, risks of malignancy in surgically-treated patients of indeterminate categories were consistently high in Eastern practice. Takezawa et al retrospectively applied the Bethesda system to their patients after triage of patients with the JTA clinical guidelines, and found that the resection rate of FN was 36.0% and the risk of malignancy was 72.4%. Furthermore, their resection rate of AUS/FLUS was 30.4% and the risk of malignancy was 88.6%, differing from rates reported from Western countries.

Conclusion: Therefore, method of patient triage has been demonstrated to be the most important deciding factor for resection rate and risk of malignancy, and the lower resection rate and high malignancy rate are achievable with proper triage of patients.

Disclosure of Interest: None declared.

SY05-3

The 2014 Italian Reporting System for Thyroid Cytology: Novel Techniques in Diagnostic Cytology

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In the period 2002–2007 several reporting systems for thyroid cytology were devised by different national and international societies in order to provide to the clinicians a tool for managing patients with nodular lesions. The Italian reporting system (IRS) for thyroid cytology has been recently updated (2014) with the introduction, on the basis of morphological criteria, of two new subcategories among the TIR3 (indeterminate) category: TIR3A (low-risk indeterminate lesion) and TIR3B (high-risk indeterminate lesion), with the purpose of decreasing the amount of unnecessary surgical procedures. In this perspective the IRS is now comparable with the most important national reporting system already published: the Bethesda System for Reporting Thyroid Cytology (TBSRTC-2008) and the British RCPath classification (2016).

The Italian reporting system has also introduced the possibility of applying new techniques which may improve the efficacy of the cytological diagnosis, in particular Liquid-Based Cytology (LBC) and Core-Needle Biopsy (CNB).

LBC is an innovative technique based on the use of a semi-automated device that has gained popularity as a method of collecting and processing both gynecologic and non-gynecologic cytologic specimens. It achieves a diagnostic sensitivity as accurate as conventional preparations (CP) especially for its excellent cell preservation and for the lack of background which decrease the amount of inadequate diagnoses. Moreover, the cellular material which has not been used for the diagnosis and is stored in the preservative solution could be effectively used for the application of immunochemical and molecular techniques. The cytologic features are similar in CP compared to LBC except for the colloid appearance as droplets in goiter and the lymphocytic infiltrate in chronic lymphocytic thyroiditis. The 2014 IRS has also included in the suggested actions for the non-diagnostic category (TIR 1) the possibility to use, in cases of repeated non-diagnostic results, the core-needle biopsy (CNB). This technique may sample thyroid nodules with a 20 to 22 spring-activated needles which obtain thin tissue samples for being processed as histological specimens. This technique has been extensively studied by several international groups with promising results. Immunochemical stains may be applied to the cellular material processed both by liquid-based cytology (LBC) as well as tissue core biopsies.

Disclosure of Interest: None declared.

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SY05-4

Reclassifying Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma: Worldwide Implications for Thyroid Cytology

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Objectives: An international panel of thyroid pathology experts has recommended that 'non-invasive' follicular variant of papillary thyroid carcinoma (NI-FVPTC) be reclassified as a 'neoplasm' rather than carcinoma. The recommended designation for this entity is NIFTP. The new classification has implications for thyroid cytology where it will likely affect the implied risk of malignancy (ROM) for the diagnostic categories of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) as well as others.

Materials and Methods: This session will review data from recent studies and discuss aspects of implementing NIFTP into thyroid cytology practice.

Results: Studies from at least 2 groups indicate that NIFTP will have the most significant impact on the ROM for the 3 indeterminate categories of TBSRTC: AUS/FLUS, Follicular Neoplasm (FN), and Suspicious for Malignancy (SM). Our multi-institutional and international collaboration (Faquin et al. 2015) found that AUS/FLUS decreased 5.3%–13.6%, FN decreased 9.9%–15.1%, and SM decreased 17.7%–23.5% when NIFTP was considered as the final histologic diagnosis. In addition to cytology, the implementation of NIFTP will also be likely to impact aspects of molecular testing, and overall effects of NIFTP will affect patient management algorithms.

Conclusion: The new NIFTP classification will have impacts on thyroid cytology classification and implications for clinical management.

Disclosure of Interest: None declared.

SY05-5

Thyroid FNA Reporting Systems and Molecular Markers: Is There a Best One?

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The latest American Thyroid Association (ATA) guidelines recommends incorporating molecular testing in the algorithms used to manage patients with indeterminate thyroid nodules and the American Association of Clinical Endocrinologists/ATA suggests that molecular testing may be considered in patients with nodules >1 cm and/or with an indeterminate result on cytology (AUS/FLUS or FN/SFN). The Bethesda and the Japanese systems for reporting thyroid cytology have no statements about the application of thyroid molecular test in fine-needle aspiration (FNA) material, while the new Italian and the new English reporting systems say that molecular test may be of utility in case of indeterminate diagnosis, without any further specification. Currently, molecular testing are used in thyroid FNA most as diagnostic tools in the indeterminate category (atypia/follicular neoplasms/suspicious for malignancy). The most promising markers seem to be *BRAF* gene mutation followed by *PAX8/PPAR* gamma translocation, having a positive predictive value (PPV) for cancer close to 100% and 80%, respectively. For *RET/PTC* translocations and the *RAS*-family mutations, the PPV value dramatically fall down, as both alterations are also detected in a variable percentage of non-malignant lesions. However, the gain in sensitivity attributable to the application of molecular tests to FNA material is around 20%, reaching 80% as opposed to 60% with cytological analysis alone. The most limiting factor in applying molecular test to thyroid FNA material is not represented by the type of material to be used and/or the type of staining used (virtually all kind of preservative and staining are usable), but by the fact that molecular alterations are present in only 60–70% of cancers. There are several possibilities for the practicing cytopathologist to run molecular tests on an aspirate: outsourcing the test (currently, the two most well known companies offering such services are Veracyte with the AFIRMA gene classifier test and Interpace Diagnostics, with the miRInform thyroid test) or using in-house facilities. In the era of personalized medicine and controlled health costs, the cytopathologist can use personalized test, as nobody better than him knows what the problematic lesions behind a diagnostic category is. For example, a diagnosis of AUS/FLUS (atypical cells of unknown significance) in the Bethesda systems showing rare clear nuclei, needs a *BRAF* test and *RAS* test, while *PAX8/PPAR* gamma and *RET/PTC* translocations are less useful, also in consideration of the paucity of material in such cases. A suspicious for a follicular neoplasm diagnosis, needs *RAS* and *PAX8/PPAR* gamma test.

In conclusion, a general consensus should be found on when and how apply molecular test to thyroid FNA in order to standardize procedures and have homogeneous and comparable results.

Disclosure of Interest: None declared.

SY06-1

Introduction for the Molecular Testing in Cytology for Lung Cancer

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Over the past years, as you know, the diagnosis and treatment of patients with lung cancer have undergone dramatic and transformational changes. Given the continuously evolving landscape in our understanding of the genetic events responsible for lung cancer pathogenesis, the integration between cytopathology and molecular diagnostics will become even more essential. Cytologic specimens, based on recent reports, represent a robust source of cellular material for molecular testing. Based on these backgrounds, we have planned this symposium. The symposium includes new WHO classification for lung cancers, clinical aspects for ROSE, application of cytologic materials for EGFR analysis,

EGFR mutation status in cell-free DNA supernatant of bronchial washings and brushings, sampling and preparation in respiratory cytology for personalized medicine, and biomarkers and personalized medicine in lung cancer. In this symposium, therefore, we think about cytologic approaches for lung malignancy together for the future development.

Disclosure of Interest: None declared.

SY06-3

The Efficiency of Histological Subtyping and Molecular Testing in Bronchoscopic Examinations for Lung Cancer Diagnosis

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In the last decade, EGFR and VEGF, and EML4-ALK have come into the limelight for decreasing tumor activities in the therapies, therefore the determination of genotypes has become indispensable for the management decision in NSCLC patients. Moreover, discrimination of histological subtypes is regarded as being very important in choosing some agents since differences in chemotherapeutic effect have been identified among the histological subtypes. Recently, a next generation immunotherapy targeting the programmed cell death 1 (PD-1) checkpoint pathway has been identified with good results in some published clinical trials as another strategy in molecular targeted therapies. Actually, an optimal specimen acquisition by bronchoscopic approaches could be difficult to get frequently, although molecular testings should include enough tumor cells in obtained samples for evaluating molecular abnormality. Rapid on-site cytopathological examinations (ROSE) have been performed with the aim of increasing diagnostic accuracy and reducing the burden of patients by reducing the number of additional procedures and aspiration biopsies during TBLB or EBUS-TBNA etc. In ROSE, each sample has been identified by whether a taken sample includes adequate cells of enough size to be diagnosed, and whether the sample includes benign or malignant cells microscopically; and might also be classified into histological subtypes on the site where possible. From July 2009 to January 2013, ROSE was performed on 106 cases at our institution, and 49 of the 106 cases were subclassified to each histological subtype as final diagnoses by pathologists (excluded 6 cases that were diagnosed as NOS, 'not otherwise specified'). In histological subtyping, the concordance rate among the histological diagnoses and cytological subtypes of the ROSE was 79.6% (39/49), in particular there were two mismatched cases between the both. More recently, an RCT aimed at evaluating the role of ROSE in EBUS-TBNA samples for molecular testing was published in 2015 (CHEST 148(6):1430–1437). Complete genotyping (EGFR and KRAS testing, followed by ALK testing for tumors with EGFR and KRAS wild-type status) was achieved in 108 of 126 patients (85.7%) [90.8% in the ROSE arm (with ROSE) vs. 80.3% in the EBUS arm, P = 0.09]. The randomized trial showed us that ROSE could prevent the need for a repeat sampling aimed at molecular testing and significantly reduce the risk in retrieving samples. In my presentation, some representative literature of studies with cytological examinations will also be interpreted for revealing optimal methods related to histological subtyping and molecular testing at present.

Disclosure of Interest: None declared.

SY06-4

Molecular Target Therapy of Lung Cancer: The Usefulness of Conventional Cytological Samples in Gene Mutation Analyses

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It is essential to validate the mutations of epidermal growth factor receptor (EGFR) in non-small cell lung carcinomas (NSCLC) before administration of tyrosine kinase targeted molecular therapy agents. NSCLC requiring EGFR gene analysis thus have gradually increased, and 146 cases have been studied in our hospital in 2014.

The analyzed materials consisted of 110 (75.3%) formalin-fixed and paraffin-embedded (FFPE) tumor samples, and 36 (24.7%) conventional cytological samples (CCS). Forty cases (27.4%) in total were positive for EGFR mutation, with 31 (28.2%) being detected with FFPE materials and 9 (25.0%) with CCS, respectively, and the positive rates were not significantly different.

FFPE materials have advantages including the availability of sections applied not only to mutation analysis but also to immunostaining *et.al.* In spite of the quantitative limitation of CCS, we encountered, however, no difficulty in detecting EGFR mutations. Furthermore, the C_T value of real-time PCR was lower when the template DNAs were extracted from CCS than from FFPE samples, implicating a better qualitative conservation of DNAs in the former and thus the consequent higher PCR efficiency.

Of the 40 positive cases, 20 harbored inframe deletion in exon 19 and 21 had L858R point mutation in exon 21, with one tumor simultaneously having both the mutations. Thirty-seven (92.5%) of the 40 tumors were adenocarcinomas.

Besides Papanicolaou stained samples, periodic acid-Schiff (PAS), Giemsa and immunostained cytological slides were also included in the present study. Although removing covering glass and getting rid of mounting Malinol were somewhat difficult, the consumed times were almost the same when either CCS or FFPE materials were used. In addition, we failed in genetic analyses with FFPE materials, but succeeded with the CCS of the same patients in several cases.

Although cytological cell collection is less aggressive than biopsy, and cytological examination with pleural effusion or cerebrospinal fluid becomes the major even the only diagnostic method in the case of recurrence, an attention should be paid to false

negative results due to the rich non-neoplastic cells contained in the cytological samples. Cytotechnologists are thus required to have the duty not only to discern malignant tumor cells on a slide, but also should have the basic moleculobiological knowledges such as DNA extraction concerning genetic analyses by using CCS.

Disclosure of Interest: None declared.

SY06-5

Epidermal Growth Factor Receptor Mutation Status in Cell-Free DNA Supernatant of Bronchial Cytology Samples

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Objectives: Lung cancer is a major cause of cancer-related mortality worldwide, and most patients have advanced disease at the time of diagnosis. Circulating free DNA (cfDNA) has potential as a liquid biopsy for monitoring cancer in real time. Elevated levels of cfDNA can be observed in cancer, particularly in advanced disease, and may also be useful as diagnostic indicators in cancer. The aim of the present study was to examine whether it was possible to detect *EGFR* mutations in cytology cfDNA (ccfDNA) from the supernatant fluids of bronchial cytology samples.

Materials and Methods: We investigated cell damage by immunostaining with cleaved caspase-3 (CC3) antibody and quantity of cell free DNA in supernatant fluid from two cancer cell lines (PC9 and H1975), and evaluated *EGFR* mutation status by PCR analysis. *EGFR* mutation was also evaluated by PCR analysis in 74 clinical samples of ccfDNA in bronchial washing samples with physiological saline, and bronchial brushing liquid-based cytology samples with CytoRich Red (Becton Dickinson).

Results: Quantity and fragmentation of cell free DNA in the supernatant fluid and cell damage and CC3 expression in the sediment gradually increased in a time dependent manner in the cell lines. In the 74 clinical samples, the quantity of ccfDNA extracted from the supernatant was adequate to perform the PCR assay, whereas the quality of ccfDNA in physiological saline was often decreased. The detection of *EGFR* mutations using ccfDNA showed a sensitivity of 100%, specificity of 89.7%, positive predictive value of 88.0%, negative predictive value of 100% and accuracy of 94.1% in samples with malignant or atypical cells.

Conclusion: Our results suggest that activating *EGFR* mutations can be detected with ccfDNA extracted from supernatant fluid of liquid-based samples by PCR assay. This will be a rapid and sensitive method of achieving a parallel diagnosis by both morphology and DNA analysis in non-small cell lung cancer patients. We next try to evaluate the DNA quality of ccfDNA in pleural fluid.

Disclosure of Interest: None declared.

SY06-6

The Specimen Sampling and Handling for Tailor Maid Treatment of the Lung Cancer

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Background: The lung cancer has been considered to be a prognostic poor cancer. In late years, molecular target drugs were developed, and it became important that I gathered an sample suitable for inspection and checked the expression of target molecules. In addition, the development of the 3rd generation EGFR-TKI advances, too, and specimen collection, in addition, suitable for an examination of gene variation, FISH and immunostaining at the time of the recurrence is important at the time of a diagnosis. It is important that I perform enough sample collection, but there are many difficult things.

Object and Method: We use navigation and a guide sheath to perform organization collection surely, but there is much that enough sample collection is difficult. There are many cases that only a specimen of the cytology was able to gather when I examine examination with bronchoscope which went in our course. In addition, I performed gene analysis with the blood and, about possibility of the gene analysis using the blood, weighed it against an operation specimen, but what a gene analyzed was difficult in the early lung cancer case. I examine the effectiveness of the gene analysis using the cytodiagnosis specimen.

Result: The immunostaining is possible in addition to gene analysis by the gene analysis being able to analyze even a cytodiagnosis specimen enough, and making a cell block.

Summary: I introduce navigation, a guide sheath and a supersonic wave bronchoscope and try certain collection. A lot of organization collection being difficult performs the analysis using the cytological specimen, and the effectiveness is shown. About the gene analysis using the blood, even the lung cancer that progressed with difficulty is limited to a part by the relatively early lung cancer.

Disclosure of Interest: None declared.

SY06-7

Biomarkers and Personalized Medicine in Lung Cancer

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Biomarkers are measurable indicators of some biological state or condition, which can be used in lung cancer for diagnostic, predictive and prognostic purposes. Exploitation of new biomarkers has always represented an open challenge for pathologists who are destined to become protagonists in the treatment of lung cancer

patients by solidifying the so-called therapeutic pathology by means of a close integration of morphology, immunohistochemistry (IHC) and molecular data. If morphology alone remains the backbone to best classify lung cancer, poorly differentiated carcinomas can be reliably subtyped for clinical purposes by approaching a minimalist antibody panel comprising of TTF1 and p40. A further improvement derives from the molecular characterization of tumors on either cytology or resection specimens for unraveling the presence of driver and/or actionable gene alterations. To this regard, multiplexed and unbiased tests by using next generation sequencing analysis have been paving the way to comprehensive molecular investigations of single patients according to the requirements of personalized medicine (the right drug, to the right patient, at the right time). Particularly relevant for the best clinical management of individual patients is the issue of intra-tumor heterogeneity (ITH), which is often encountered in lung cancer where multiple sub-clones are destined to spatially and temporally evolve, either spontaneously or under therapy, leading to the development of secondary resistance mechanisms. Organizing mutations into phylogenetic trees by dissecting their prevalence and distribution in different areas within the same tumor mass allows the complex molecular landscape of tumors to be interpreted in terms of dominant or trunk mutations if they are present in most sub-regions of tumors, branching mutations if present in at least two regions of tumors and private mutations if confined to a single tumor region. A direct implication of such phenomenon is that the same mutation is able to act differently in diverse tumors, indicating that a variety of molecular mechanisms is actually underlying the development of lung cancer. All these findings sustain the current operational view according to which the pathology report informs significantly the therapy choice by integrating improved diagnoses, IHC findings and molecular data to realize more robust and reliable models of patients' clinical management.

Disclosure of Interest: None declared.

SY06-8

Molecular Testing in Cytology for Lung Cancer: New Aspect of Lung Cancer Diagnosis

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Due to the advance in molecular medicine, the biological behavior of lung cancer is evaluated both by morphology and by genetic analysis. Histologic type and stage are still essential to decide the treatment strategy of each patient but the chemotherapy regimen is personalized based on the genetic abnormality. Histological classification of lung cancer has been revised and prognostic aspect is highly evaluated. Immunohistochemical staining is routinely used to diagnose the histologic subtype and reduce NOS cases.

Sampling is also important to obtain high quality materials sufficient for microscopic and genetic examinations and comprehensive interpretation of results of these examinations is essential for the optimal management for lung cancer patients. In this session, the state of the art of lung cancer diagnosis is diucussed.

Disclosure of Interest: None declared.

SY07-1

Roles of EUS and EUS-FNA in Diagnosing Pancreatic Malignancy

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Pancreatic ductal adenocarcinoma (PDAC) is the most lethal type of gastrointestinal cancer with a five-year survival rate of 5%, yet it remains an unresolved but significant therapeutic challenge. The aggressive features of PDAC include an insidious presentation, early involvement of major vessels precluding resectability, debilitating symptoms at the late stage, and *de novo* chemo-resistance.

However, the five-year survival rates of patients with International Union against Cancer (UICC) stages 0 and 1a are 85.8% and 68.7%, respectively, according to the Japan Pancreatic Cancer Registry.

Because it plays an important role in improving the overall survival of patients with PDAC and thus all effort should target early diagnosis and the reliable identification of patients who will benefit from major surgical intervention.

While CT should be the first choice of modality to assess patients with suspected PDAC, EUS is more accurate, especially for detecting lesions <10 mm. The roles of EUS and EUS-FNA are crucial because accurate preoperative evaluation is essential to select appropriate management strategies. The sensitivity of EUS-FNA is 75%–90%, with virtually 100% specificity for solid pancreatic tumors. The addition of molecular genetic analysis to determine for example, KRAS mutations to cytological or histological analyses might improve sensitivity. However, rapid on-site evaluation (ROSE) is mandatory to improve the sensitivity and specificity of EUS-FNA. I would like to describe the advantages of ROSE for pancreatic tumors.

Stage 0 (carcinoma in situ) PDAC has recently been discovered uncommonly. Stage 0 PDAC cannot be diagnosed by EUS-FNA. Only endoscopic retrograde pancreatography (ERP)-cytology can detect Stage 0 PDAC. Nasopancreatic tube drainage of pancreatic juice for cytodiagnosis has recently become more frequent in Japan. I will also introduce this method.

Disclosure of Interest: None declared.

SY07-2

EUS Guided FNA of Pancreas: Experience at a Tertiary Care Centre

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Endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA) is currently performed on a routine basis at many tertiary care centers and it is evident that this procedure has a major impact on the therapeutic management of patients by obtaining a definite tissue diagnosis from lesions outlined by endosonography.

The reported yield of EUS-FNA is about 90–95%, with an overall sensitivity and specificity of 90% and 100%, respectively.

EUS guided FNA is a minimally invasive procedure used to obtain definitive tissue diagnosis from the lesions outlined by endosonography. Minute lesions up to 5 mm can be biopsied with this technique.

Since its introduction in the 1980s, endoscopic ultrasound (EUS) has become established as an accurate imaging procedure in the diagnosis and staging of pancreatic tumours. In patients with operable conditions, EUS is a third line investigation after ultrasound and spiral computed tomography (CT) scan, where these investigations have not detected obvious criteria for irresectability. A combination of EUS and EUS-FNA in patients with pancreatic tumours allows staging and the possibility of a diagnosis and immunophenotyping during the same procedure.

Another advantage of EUS-FNA is its low-complication rate. According to recent American Society of Gastrointestinal Endoscopy (ASGE) guidelines, the complication rate of EUS-FNA is 1–2%, with virtually no severe or fatal complications. There is also considerable evidence that EUS-FNA in experienced hands can replace many other far more invasive procedures, including surgical biopsy.

Rapid on-site evaluation (ROSE) of slides obtained by EUS-FNA, carried out by a trained cytopathologist, helps in improving the yield, and guides the gastroenterologist in obtaining samples for advanced ancillary studies such as immunophenotyping, flow cytometry, fluid chemistry or molecular analysis, as well as for culture, all of which may enhance the diagnostic accuracy of the procedure.

Between 2012–2015, a total of 322 EUS-FNA were performed at SKMCH & RC, a tertiary care cancer centre in Lahore, Pakistan. Pancreatic and peripancreatic masses represented 39% of these EUS-FNA procedures. The major challenges associated with EUS-FNA are low cellular yield, material inadequacy and distinguishing between reactive and neoplastic cells. The sensitivity and specificity of EUS-FNA at SKMCH & RC is over 90%.

Ancillary techniques, including immunophenotyping, flow cytometry and fluid chemistry are routinely performed on the samples obtained. Immunohistochemistry is performed on cell block as well as smears.

Disclosure of Interest: None declared.

SY07-3

Pancreatic Cytopathology: The Hopkins Experience

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The lecture will focus on diagnostic issues of practical importance and will describe the potential pitfalls and limitations leading to erroneous diagnosis on EUS-guided FNA of this difficult anatomic site. The importance of a multidisciplinary approach when dealing with pancreatic aspiration specimens will be highlighted as well as the clinical implications of FNA interpretations. Emphasis will be placed on the role of ancillary studies particularly immunostaining in difficult to classify pancreatic tumors. Mimics of cancers such as chronic pancreatitis will be particularly discussed to avoid misdiagnoses.

Disclosure of Interest: None declared.

SY07-4

Cytology of Intraductal Papillary Mucinous Neoplasms (IPMNS)

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Introduction: Intraductal papillary mucinous neoplasms (IPMNs) of the pancreas have four histological subtypes whose malignant potential are different from one another. Preoperative diagnoses of the IPMN subtypes and dysplastic grades are expected, but it is difficult to diagnose them by image findings alone (CT, MRI, etc.). According to the International Consensus Guidelines 2012 for the management of IPMN and MCN of the pancreas, cytological diagnosis plays an important role for treatment strategies of IPMNs. Especially, in BD-IPMN with cytological findings of high-grade dysplasia, surgery would be considered if clinically appropriate. However, cytological features of subtypes and dysplastic grades are not yet confirmed. In this study, we investigated cytological characteristics of the IPMN subtypes (especially, gastric and intestinal subtypes) and cellular dysplastic grades (especially, intermediate and high grade).

Methods: 16 patients with IPMN who underwent resection of the pancreas lesions, were included in this study. Histological subtypes were determined based on the immunohistochemical staining for MUC1, MUC2, MUC5AC in the resected specimens. Preoperative pancreatic juice cytology slides were reviewed to evaluate subtype and cellular dysplasia.

Results:

Gastric subtype. The neoplastic cells are arranged in low papillary clusters and sheet-like cell clusters with oval nuclei and mucinous background. As dysplastic grade advances, cell clusters become smaller and nuclear atypia become great.

Intestinal subtype. The neoplastic cells are villous papillary and sheet-like clusters with oval to cigar shaped nuclei. As dysplastic grade advances, mucin-containig columnar cells decreased, irregular arranged cluster margins and nuclei atypia become great.

Pancreatobiliary subtype. The neoplastic cells are small and a few mucin-containig columnar cells are exist.

Conclusion: Cytological features would be a good predictor of histological subtypes and dysplastic grades of IPMNs.

Disclosure of Interest: None declared.

SY07-5

Solid Pancreatic Lesions: An Algorithmic Approach to Diagnosis

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Learning Objectives: Pancreatic cytology is one of the more challenging sample type for a cytopathologist.

At the end of the session participants will:

1. Learn a simple easy to follow algorithm to approach various solid pancreatic lesions.

2. Recognize salient features that distinguish chronic pancreatitis from pancreatic adenocarcinoma.

3. Recognize salient features that distinguish Pancreatic Endocrine neoplasm from Solid Pseudo papillary neoplasm of the pancreas.

4. Understand salient ancillary studies that may help distinguish various solid pancreatic lesions.

Pancreatic cytology is one of the more challenging sample types that a cytopathologist encounters. This session will utilize a simple easy to follow algorithm highlighted in one of the most utilized book by endosonographers. The session will utilize cases as a jump board to highlight salient cytologic features to distinguish chronic pancreatitis from pancreatic adenocarcinoma and solid pseudo papillary neoplasm of the pancreas from pancreatic endocrine neoplasia. In the process this session will also demonstrate how judicious use of ancillary study can help distinguish these challenging lesions.

Disclosure of Interest: None declared.

SY08-1

New Horizons in Cervical Cancer Screening: Combining Molecular and Cytology Based Technologies to Provide a Solution for Future of Cervical Screening

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Cervical screening is rapidly changing face in these years. Accumulated evidence from more than a decade of clinical randomized control trials, split sample trials, and implementation has proven superior cervical cancer protection by molecular screening for human papillomavirus compared to cytology based screening for cellular abnormalities. On top of this, molecular HPV screening allows for longer screening intervals for HPV negative women to the benefit of the women and with a cost saving for the screening programs. Countries like Holland, Sweden, Norway, Scotland, England Denmark and others are currently underway with national recommendations fully or partially replacing cytology with primary molecular HPV screening. Most recently, in September 2015, the European Guidelines for cervical screening also called for primary HPV screening rather than cytology.

The four principal challenges driving the change from cytology to molecular HPV testing are:

- The well-established superior sensitivity for ≥CIN2 of molecular HPV DNA testing compared to cytology
- The ability to offer HPV testing from self-collected samples to non-attenders
- Risk stratification by molecular HPV technologies will re-focus efforts on women at risk, individualizing the screening, and
- The introduction of HPV vaccinated women in cervical screening requires adaptation, as vaccinated birth cohorts will substantially decrease cytology sensitivity.

However, are all molecular HPV assays 'created equal'? Will knowledge of a given woman's HPV genotype add value in risk stratification for follow up? And how do we deal with the many screening false positive HPV tests? How will HPV vaccination change the requirements of screening, and how can primary HPV screening and self-sampling to otherwise screening non-attenders be combined to offer a 21th century screening solution. Finally, will molecular biomarkers like methylation come into play in order to optimize molecular HPV cervical screening, eventually resulting in purely molecular screening?

To answer these questions, the lecture will review the changes and implementation of HPV testing in the organized general population-based cervical cancer screening program in Denmark, combining HR-HPV testing with HPV genotyping, liquid based cytology, and HPV sample self-collection. All elements included to form a consolidated, effective cervical cancer screening solution for the future.

Disclosure of Interest: Dr. Jesper Bonde has in the past served as paid advisor to Roche and Genomica, and has received honoraria from Hologic/Gen-Probe, Roche, Qiagen, Genomica, AxLab and BD diagnostics for lectures. He is principal investigator of studies funded by BD diagnostics, and the principal investigator on studies co-funded by Roche, Qiagen, Genomica, BD Diagnostics and GenProbe.

SY08-2

Cervical Screening in the UK: A Triumph and a Tragedy?

Amanda Herbert

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Introduction: When Leo Koss coined this phrase the situation was entirely different: high expectations of successful annual screening in the US marred by lapses in quality control [1]. In the UK now QC is excellent, organised 3–5 yearly screening has been highly successful in reversing an increased risk of disease and 80% of teenage girls have been vaccinated since 2008. The 'tragedy' is that incidence has more than doubled in women aged 25–29 years, and screening coverage has fallen to around 60% in that age group while background risk of disease has increased again.

Advantages and Challenges of New Screening Protocols: Rather than 'high expectations', cytology in the UK is often assumed to be less effective than HPV testing, which is likely to be

introduced nationally as a primary test. Many of us, including the response in consultation from the British Association for Cytopathology (http://www.britishcytology.org.uk/resources/BAC-NSC-Response-2015.pdf), have reservations about the HPV test alone without cytology backup in the first high-prevalence rounds of screening in women from age 25 who have never been screened before.

Co-testing limited to the first two high-prevalence rounds of screening would optimize sensitivity while substantially reducing the proportion of negative tests examined cytologically, which would be necessary in low-risk predominantly vaccinated population. Additionally, limited co-testing would allow HPV-negative samples to be included in the slides examined: their complete absence would compromise quality control [2].

Anxiety about the effect of surgical excision of CIN may deter medical practitioners and women from cervical screening; but the adverse affects are due to deep excisions, more likely to be needed for recurrent or widespread lesions. Initial surveillance rather than immediate treatment of young women with CIN2 might allow treatment to be limited to lesions at higher risk of progression. The LAST guidelines for using p16 in this context may help – but a simple distinction between high-grade and low-grade could lose the 'buffer zone' of intermediate lesions between low-grade lesions likely to be reversible and the robust diagnosis of CIN3 with its known risk of progression.

Conclusion: Accurate screening will be needed for unvaccinated women and non-16/18 HPV lesions. Declines in rates of cervical cancer and its precursors due to vaccination will mask these rates in women still at risk – and they should not be forgotten.

Disclosure of Interest: None declared.

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SY08-3

Using HPV Testing to Improve Cervical Cancer Screening: Cotesting Versus HPV Primary Screening

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Pathology and Cell Biology, Columbia University, United States

Objective: Incorporating HPV testing increases the sensitivity of cervical cancer screening. However, it is unclear in which settings we should combine HPV testing and cytology (cotesting) and in which HPV testing alone (HPV primary screening) would be preferred. There also remain questions as to the best approach to managing HPV(+) women.

Methods: Data from large screening trials including the ATHENA study will be reviewed.

Results: Almost all large screening trials have reported that screening with HPV detects more women with high-grade cervical neoplasia (CIN2+) than does cytology alone, provided HPV(+) women are followed-up. In the Canadian FOCAL trial women were randomized to either cytology with reflex HPV testing for ASC-US or HPV with reflex cytology for HPV(+) women and repeat HPV testing at 6–12 mos if reflex cytology is NILM. At baseline, detection rates of CIN2+ and CIN3+ were similar in both arms. However after repeat HPV testing of HPV(+) women with NILM, the HPV arm had increased CIN2+ detection (16.1 per 1000) compared to cytology arm (11.0 per 1000). In the U.S. ATHENA study cytology with reflex HPV testing for ASC-US detected 144 cases of CIN2+ in women at baseline which was identical to the number detected with cotesting. However, HPV primary screening with referral of all HPV 16/18(+) women to colposcopy and reflex cytology for HPV(+) women with other HPV genotypes detected 178 cases ($p < 0.05$). After repeat HPV testing of HPV(+) women with initial NILM cytology, both cotesting and HPV primary screening detected 299 cases.

Conclusion: Both cotesting and HPV primary screening increase the detection of CIN2+ compared to cytology alone.

Disclosure of Interest: Consultant and speaker for Roche and BD Diagnostics.

SY08-4

Recent Progress in Cervical Cancer Screening and Bethesda System in Japan

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'Japanese Society of Clinical Cytology' was set up in 1961, since then cervical cytology for cervical cancer screening started in Japan, and it was followed by officially registered cervical cancer screening system in 1983. Thanks to this screening system, the early stage detection of cervical cancer increased dramatically. In light of this system, the number of deaths related to invasive cervical cancer in Japan was reduced. However, screening rate is still low (about 20–25%), compared to United States, and whish is over 80%.

'The Bethesda System (TBS)' for reporting cervical cytology started in 1988 at the NIH in Bethesda, Maryland. The objective was to establish standardized terminology and management. By early 2003, over 85% of laboratories in United States had implemented Bethesda 2001 terminology, and the adoption of TBS in the international cytopathology community had produced a significant impact.

In Japan, this TBS atlas was translated to Japanese in 2007, and introduced to Japan in 2008. At first 'Pap Class' system had been used in reporting terminology of cervical cytology, and it was followed by TBS 2001 terminology in 2008. Leaving behind United States by more than 5 years, Japanese cervical cancer screening system implemented TES 2001 terminology.

I am going to explain the current state and the issues regarding the Japanese cervical cancer screening with TBS 2001 as follows.

Firstly, the methods of screening; current state of HPV co-testing, and conventional or liquid-based preparations. Secondly, the

management and follow-up system after screening. So far, there is no evidence-based consensus guideline for management and follow-up, which has the fundamental principles of balancing harms and benefits and providing equal management for equal risk. Finally, I am going to explain current issues of Bethesda 2001 terminology, especially atypical squamous cells and atypical glandular cells (include endometrial cells).

Disclosure of Interest: None declared.

SY09-1

Evolution of the Translational Potential of New Technology in the Field of Cytopathology

Koji Tsuta

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After the discovery of crucial 'driver' oncogenic gene alterations such as that in the epidermal growth factor receptor gene mutation in non-small cell lung carcinoma, histological and/or cytological diagnoses for malignant tumors has changed. Cytological diagnoses are required not only to judge whether the lesion is malignant or benign but also to decide on the type of therapy. This extremely customizable approach is expected to maximize treatment efficacy for patients with malignancies. Molecular tests have enabled objective and reproducible testing of tumors, which is of high clinical relevance while selecting a target therapy. Although cytological features are tightly correlated with genetic alterations, such as signet-ring cell morphology in non-small cell lung carcinoma and translocation in anaplastic large cell lymphoma kinase gene, these can never be better than molecular diagnostics. In addition, although cytological samples can be used in molecular tests, the majority of molecular testing carried out on histological samples.

Thus, the era of molecular diagnostics is seen by some as a threat to cytopathology. What are the merits of cytopathology? Cytological samples have been shown to be suitable for use in a wide range of molecular tests. In addition, it is important that molecular results be interpreted with consideration of cytomorphology for a final diagnosis with high clinical value. In this presentation, I shall introduce the integration of the molecular diagnostics with cytology.

Disclosure of Interest: None declared.

SY09-2

The Role of Cytotechnologists in Molecular Diagnostics

Michelle Herzog

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Traditionally, cytotechnologists screen and evaluate Pap tests and non-gynecological cytology specimens.

With the recent advances in personalized medicine, the demand for molecular tests continuously grows, creating new opportunities for cytotechnologists.

Importantly various molecular tests can easily be performed on conventional cytological specimens. The adequacy of the specimen and the training/experience of the cytotechnologist are essential for good results of any molecular test. Because of their high level of expertise both in morphology and in various technical methods, cytotechnologists are particularly well suited to be involved in molecular analyses.

At our institution the role of cytotechnologists has changed progressively over the years. In addition to the classical skills of routine screening, on-site evaluation of FNA specimens and education of junior staff, cytotechnologists are more and more involved in different molecular ancillary techniques such as immunocytochemistry, flow cytometry, HPV-testing, laser capture microdissection for gene sequencing and fluorescence in situ hybridization (FISH). At the beginning, these activities were 20 to 40% of our workflow. Currently, they are a full-time job for some of us.

Some cytological specimens, in lung cancer cytology for example, require very precise pre-analytical tasks prior to the analysis. Detection of predictive molecular markers, like EGFR or KRAS gene mutations and ALK or ROS1 rearrangements, require the combination of different technologies (gene sequencing and FISH). To perform both tests on one single specimen a close collaboration between cytopathologists und cytotechnologists is necessary.

In the era of molecular based medicine, the practice of cytotechnologist is a rapidly evolving field which has enhanced the role of cytopathology.

Disclosure of Interest: None declared.

SY09-3

A Novel Highly Sensitive and Specific Flow Cytometry System for Cervical Cancer Screening

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Purpose: This study assessed the performance of a novel flow cytometry (FCM) cervical cancer screening system compared with human papillomavirus (HPV) Hybrid Capture 2 (HC2).

Methods: Chinese women aged 20 years or older were enrolled in this study at Fudan University Shanghai Cancer Center. All participants underwent cytology/pathology testing (gold standard), HPV HC2 testing and FCM testing involving analysis of cell proliferation index (CPIx).

Results: Among 437 women enrolled in this study, 185 women (42.3%) were diagnosed as 'gold standard positive' by pathology with diseases including cervical intraepithelial neoplasia (CIN) grade 2 (n = 11), CIN3 (n = 41), squamous cell carcinoma (SCC; n = 115), adenocarcinoma in situ (n = 2) and adenocarcinoma (n = 16). The remaining 252 cases were deemed 'gold standard negative'. The sensitivity was 87.6% (95% CI, 82.8–92.3) for FCM testing and 89.7% (95% CI, 85.4–94.1; p = 0.5121) for HPV HC2 testing. The specificity of FCM testing was 90.5% (95% CI, 86.2–94.7), which was superior to the specificity of HPV HC2 testing (84.5%, 95% CI, 79.3–89.7; p = 0.04). In the 20–29 years old group, the sensitivity and the specificity of FCM testing were 90.0% (95% CI, 71.4–100.0) and 92.9% (95% CI, 76.9–100.0), respectively. The FCM testing CPIx statistically increased with the transition from normal cervical specimens to SCC specimens.

Conclusions: Our results showed that the FCM screening system had high sensitivity and specificity for women of various ages. The FCM CPIx was able to evaluate the severity of disease quantitatively.

Disclosure of Interest: XiaohuaWu has received research grant from Sysmex Corporation. The otherauthors declare that they have no competing interests.

SY09-4

Lymph Node FNA and Ancillary Techniques: What Can Be Done?

Pio Zeppa

University of Salerno, Italy

Lymph node (LN) fine-needle aspiration (FNA) is a generally accepted procedure in the diagnosis of LN enlargements whereas the FNA diagnosis of lymphoma is still controversial. Nonetheless, meanwhile lymphoma diagnosis has become less 'histological' and increasingly phenotypical and molecular, cytopathologists have learned to apply the same ancillary techniques that are used on tissues on FNA samples. As a result FNA may be successfully used to diagnose and sub-classify much of non-Hodgkin lymphoma (NHL). The main task of LN FNC, as first line diagnostic procedure, is clonality assessment. Clonality can be evaluated by the phenotypic quantification of light chain, mainly by flow cytometry (FC) and may be suggested by specific phenotypic profiles, such as CD5/CD19, highest percentage of CD10/CD19 co-expression or loss of one of T-cell surface antigens CD2/CD3/CD7. Nonetheless a definite number of the cases lack these phenotypic profiles and some cases do not show light chain restrictions or lack of their expression. Clonality on FNA can be also determined by FISH evaluation of the human immunoglobulin heavy-chain (IGH) using a split-signal IGH probe. IGH locus is frequently involved in different translocations of NHL, and the detection of any breakage involving the IGH locus identifies a B-cell NHL. FISH can also detect specific abnormalities, such as the t(14,18)(q32;q21) in follicular lymphoma (FL) or the t(11;18)(q21;q21) in MALT. Nonetheless chromosomal abnormalities are usually investigated when a specific NHL is suspected or has already been diagnosed rather than determine LN FNA clonality. With reference to molecular testing, immunoglobulin/T-cell receptor (IG/TCR) PCR assessment is the main specific procedure. IG/TCR PCR clonality can be determined on genetic material obtained from fresh or frozen cells whereas different samples, such as paraffin-embedded tissues, smears or fluids are optimal samples too. Moreover 26 new genes have been recently identified as contributors to NHL based on their mutation patterns. The identification of these mutations by new generation sequencing (NGS), might be performed on FNA samples providing better diagnoses, accurate prognostic information and information for targets of therapy. In this perspective FNA samples are excellent but are generally scanty, therefore the disposal, the preservation and the storage of genetic material is an important point in the pre-analytical phase of LN FNA. The immediate evaluation of LN-FNA by rapid on-site evaluation (ROSE) allows to evaluate the adequacy of the smear, the diagnostic orientation, the disposal of additional passes for specific ancillary techniques and the amount of necessary material. Therefore LN FNC ROSE and an accurate management of FNA material is mandatory in LN FNA.

Disclosure of Interest: None declared.

SY10-1

Gene Aberrations for Precision Medicine of Lung Cancer

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Lung adenocarcinoma (LADC), the most frequent histological type of lung cancer, is often triggered by an aberration in a driver oncogene in tumor cells. Examples of such aberrations are *EGFR* mutation and *ALK* fusion. LADC harboring such mutations can

be treated with anti-cancer drugs that target the aberrant gene products. Additional oncogene aberrations, including *RET, ROS1,* and *NRG1* fusions, skipping of exon 14 of *MET*, and mutations in *BRAF, HER2, NF1*, and *MEK1*, were recently added to the list of such 'druggable' driver oncogene aberrations, and their responses to targeted therapies are currently being evaluated in clinical trials [1, 2].

On the other hand, about 30% and 50% of LADCs in patients in Japan and Europe/USA, respectively, lack the driver oncogene aberrations listed above. In addition, small cell lung carcinoma (SCLC), another deadest type of lung cancer, lacks such aberrations, either. Therefore, novel therapeutic strategies, such as those that exploit the vulnerabilities of cancer cells with non-oncogene aberrations, are urgently required. We have proposed 'Paralog Targeting Therapy' to specifically kill cancer cells with deficiency of a gene by inhibiting its functional paralog [3]. About 10% of SCLCs have deficiency in *CBP/CREBBP*, encoding a histone acetyltransferase (HAT) [4]. Here, we present a novel therapeutic idea to kill such cancer cells by inhibiting activity of p300, a functional paralog of CBP, by a specific HAT inhibitor or bromodomain inhibitors [5]. We discuss here the promise of paralog targeting therapy in cancer clinic.

Disclosure of Interest: A research grant from Daiichi-Sankyo Co Ltd.

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SY10-2

A Non-Invasive Diagnosis of Ovarian Clear Cell Carcinoma

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Objectives: Circulating tumor DNA (ctDNA) analysis constitutes a non-invasive approach to diagnose cancer. CtDNA is fragmented to an average length of 140 to 170 bp and is present in only a few thousand amplifiable copies per milliliter of blood, of which only a fraction may be diagnostically relevant. Recent progress in PCR technology enabled to detect somatic mutations characteristic of ovarian cancers by measuring small amount of DNA such as ctDNA and/or Pap smear derived DNA (psDNA). The aim of this study was to establish a non-invasive diagnosis procedure of ovarian clear cell carcinoma (CCC) by evaluating ctDNA and psDNA patients.

Materials and Methods: Comparative genomic hybridization (CGH) array profiles of 144 CCC samples was conducted to identify gene locus which are frequently amplified/deleted in patients. We prospectively recruited 26 patients with histologically confirmed ovarian CCC from 2010 to 2015. Digital PCR (dPCR) was applied to examine the gene amplifications and/or mutations of their ctDNA and psDNA.

Results: We identified genomic regions which are highly amplified in CCC patients on chromosome 8 (region1~3), chromosome 12, and chromosome 20. Kaplan-Meier progression free survival estimates showed relatively poor prognosis for patients with amplification of region2 and/or 3. Univariate analysis showed that patients with amplifications of more than two genomic regions correlated with poor survival. We also succeeded to detect 4 genomic regions from ctDNA of CCC patients by dPCR. Furthermore, using the same analysis, we detected PIK3CA and KRAS mutations which are commonly reported in CCC.

Conclusion: We could successfully demonstrate that our non-invasive dPCR-based diagnosis can detect gene amplification and/or mutation in ctDNA/psDNA from CCC patients. This method will enable to diagnose the early stage of CCC as well as to monitor the therapeutic effect of drugs and detecting cancer recurrence and metastasis.

Disclosure of Interest: None declared.

SY10-3

Novel Strategy for Controlling the Growth of Small Cell Lung Cancer

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Small cell lung cancer (SCLC) is the most aggressive type of lung cancer, showing a typical clinical feature of early metastasis and resistance to second-line therapies after disease recurrence. Therefore, only 5% of SCLC patients survive beyond 5 years after diagnosis. More importantly, the prognosis has not been improved over the last three decades. This could be in part due to the fact that proper targeting therapies for SCLC have not yet been developed. Recent studies have indicated that products from activating oncogenes are proper targets for therapies in several human cancers. Therefore, for the improvement of patients' outcome in this disease, it is important to identify druggable targets activated by genetic alterations in SCLCs and to develop a novel therapeutic strategy controlling the growth of SCLC cells by manipulating the activities of activated oncogene products. For this reason, several different types of genome-wide analyses have been performed by us and others to identify genes targetable for therapy in SCLC patients. However, only a limited number of genes, such as *TP53* and *RB1*, have been shown to be recurrently mutated with high frequencies in SCLCs. The most prominent activating oncogene alteration in SCLC was amplification and overexpression of one of *MYC* family genes, *MYC*, *MYCL*, and *MYCN*, which occurs in approximatively 20% of SCLCs in a mutually exclusive manner. Since c-Myc, L-Myc, or N-Myc protein is overexpressed in SCLC cells

with amplification of the respective *MYC* family gene, we hypothesized that Myc family proteins could be valuable targets for therapy in SCLC patients. However, it has been suggested that inactivation of *TP53* and/or *RB1* could interfere with the therapeutic approaches based on Myc targeting. Therefore, we investigated the effects of inhibiting the activities of *MYC* family gene products on the growth of SCLC cells. Up to now, we have obtained the evidence of growth suppression by controlling the activities of amplified and/or expressed *MYC* family gene products in SCLC cells by using a dominant negative MYC construct, short hairpin RNAs and a MYC specific inhibitor. It was noted that c-Myc inhibitors were also effective to inhibit L-Myc and N-Myc activities in SCLC cells resulting in the growth suppression of *MYCL* or *MYCN* amplified SCLC cells. The results indicate that a common inhibitor for three Myc family proteins will be highly useful for the treatment of SCLCs. Therefore, we are currently investigating the molecular pathways of growth suppression in SCLC cells, in which two critical negative cell cycle regulators, *TP53* and *RB1*, are commonly inactivated by genetic alterations. In this congress, we will present our current data for controlling the growth of SCLC cells by manipulating the MYC activities using several different strategies.

Disclosure of Interest: None declared.

SY10-4

Clinical Implications of Fluorescence-Emitting Virus Guided Peritoneal Cytology in Gastrointestinal Cancer

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Background: Peritoneal washing cytology (CY) is used in the diagnosis and staging of gastrointestinal cancers. Because positive CY is implicated to be incurable especially in gastric cancer, it is critical factor to determine the way of treatment. Although conventional cytology is still the most reliable diagnostic modality, there might be room for improvement. We developed a new approach to visually capture live cancer cells using a green fluorescent protein (GFP)-expressing attenuated adenovirus, in which the telomerase promoter regulates viral replication (TelomeScan). The study to evaluate the feasibility of this virus-based imaging cytology in the clinical samples is under way.

Methods: Peritoneal cytology is routinely performed in patients with advanced gastric cancer. About 50 ml of the lavage fluid was subjected to conventional cytology, and the residual fluid was used in this study. The cells in the fluid was infected with TelomeScan at 1 multiplicity of infection for 24 hours, and the fluorescence was observed and the number of positive cells was counted. The samples obtained from 67 patients were analyzed in this study.

Results: Our biological capturing system can image intraperitoneal cancer cells with telomerase activities as GFP-positive cells. GFP-positive cells could be detected in 25 cases. There was some concordance between conventional cytology and virus-based cytology. The cases detected GFP-positive cells showed a significant worse prognosis when compared to the negative cases, especially in the cytology positive cases.

Conclusions: Using the virus 'TelomeScan', we were able to detect cancer cells as GFP-positive cells in peritoneal lavage fluid from gastric cancer patients. The presence of GFP-positive cells in peritoneal wash was associated with worse prognosis. The virus-guided cytology test may have clinical implication as prognostic biomarkers in gastric cancer.

Disclosure of Interest: None declared.

SY11-1

Utility of p16/CDKN2A FISH and BAP1 Immunohistochemistry in Distinguishing between Mesothelioma and Reactive Mesothelial Proliferation

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Objectives: Mesotheliomas often present with serosal effusions, which are submitted for cytological evaluation. However, there is doubt as to the ability of the cytopathology to establish a definitive diagnosis of mesothelioma because of morphological overlap between malignant and benign mesothelial proliferation. There is no immunohistochemical staining that allows definite separation in this setting. Fluorescence in situ hybridization (FISH) of *p16/CDKN2A* is helpful for this differentiation. Recently, loss of BAP1 expression has been reported in mesotheliomas. The aim of this study was to confirm the clinical applicability of p16 FISH and BAP1 immunohistochemistry for the cytological diagnosis of mesothelioma.

Materials and Methods: Twenty-one cell blocks of serosal effusions with atypical mesothelial cells from patients who had been histologically confirmed to have non-sarcomatoid pleural mesothelioma between 1995 and 2015 were analyzed. The cell blocks and biopsy samples of the tumor of the same patients were analyzed with p16 FISH and IHC with BAP1. We also analyzed cell blocks of the 17 cases with reactive pleural effusion. FISH analysis on cell blocks was followed by immunofluorescence with epithelial membrane antigen (EMA) to highlight the mesothelial cells.

Results: Clusters of atypical cells were observed in 90% (19/21) of the cell blocks. Ten cell blocks contained large clusters (more than 0.1 mm), and nine contained small clusters (less than 0.1 mm). We set the cut-off value of homozygous deletion of *p16/CDKN2A* as 15% from the analysis of reactive mesothelial cells. Homozygous deletion of *p16/CDKN2A* was observed in 76% (16/21) and BAP1 loss was observed in 89% (16/18). Homozygous deletion of *p16/*

CDKN2A and/or BAP1 loss was observed in 100% (18/18) of cell blocks. All cell blocks (16/16) from the patients with mesothelioma tumor with homozygous deletion also harbored homozygous deletion in atypical mesothelial cells. Concordance of the results of BAP1 loss between surgical biopsies and cell blocks of mesothelioma was also good. Most of the clusters with homozygous deletion were positive for EMA, but some were negative.

Conclusion: The presence of large clusters of atypical cells may be characteristic of mesothelioma. Immunofluorescence helps to identify the mesothelial cells in FISH analysis. We confirmed that the results of p16 FISH and BAP1 immunohistochemistry on cell blocks are as reliable as on tissue sections. However, additional confirmation of the mesothelial origin of atypical cells with immunohistochemistry is needed for the cytological diagnosis of mesothelioma.

Disclosure of Interest: None declared.

SY11-2

Immunocytochemistry in the Work-Up of Mesothelioma and Its Differential Diagnosis

Claire W. Michael

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Introduction: Reviewing a cellular effusion sample, the pathologist has to answer a set of questions in order to achieve an accurate diagnosis. Such questions include: 1) Are these cells mesothelial or epithelial? 2) If mesothelial are they benign or malignant? 3) When malignant, are they mesothelial or a mimicker such as adenocarcinoma, squamous cell carcinoma and urothelial carcinoma?

Methods and Results: Immunocytochemistry can be a great compliment in addressing the above questions and arriving at a definitive diagnosis.

Several markers including desmin, EMA, GLUT-1, IMP3, Beta catenin, Ki-67 and P53 have been reported to help in distinguishing reactive from malignant mesothelial cells with variable sensitivities and specificities.

Markers useful in detecting mesothelial origin include D2–40, podoplanin, Calretinin, mesothelin, CK 5/6 and WT-1.

Markers useful in identifying epithelial origin include CEA, MOC-31, Ber Ep4, Leu-M1 (CD 15), B 72.3, and BG-8.

Squamous cell carcinoma expresses most epithelial markers in high percentages however they are characteristically negative for WT-1. They are immunoreative for CK 5/6, P63 and P-40 with the latter having the best sensitivity and specificity. Urothelial carcinoma tend to be non-reactive to most epithelial and mesothelial markers and would focally react to CK 5/6 and P-63.

Conclusion: In general it is recommended that at least two mesothelial and two epithelial markers be used as a panel for the initial screening of the cells. If one of the above mimickers is suspected, additional markers can be added to the panel as indicated.

Disclosure of Interest: None declared.

SY11-4

Clinical, Cytological, and Molecular Biological Approaches to the Diagnosis of Early Mesothelioma

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Malignant pleural mesothelioma (MPM) is a refractory tumor with poor prognosis associated with asbestos exposure. Pleural effusions are frequently developed at early stages of MPM, and cytological analysis of pleural effusions is useful to identify patients with MPM. Recently, we proposed a term of radiological T0-MPM for the early stage in which pleural effusion is observed but neither apparent tumor nor pleural thickening are detected on chest X-ray and CT and no abnormal uptake is found in ^{18}F-fluorodeoxyglucose positron emission tomography. Radiological T0-MPM potentially includes not only thoracoscopic T0 MPM in which no apparent tumor is macroscopically visible in thoracoscopy, but also histological early mesothelioma in which mesothelial proliferation is localized on the serosal surface of parietal pleura or limited to submesothelial fibrous tissues of parietal pleura.

Recently, genetic abnormalities and molecular markers, which can discriminate MPM from reactive mesothelial hyperplasia/proliferation (RMH), have been reported. The *CDKN2A* (*p16^{INK4a}*/*p14^{ARF}*) gene at chromosome 9p21 is frequently deleted in MPM. Fluorescence in situ hybridization (FISH) analysis using locus-specific *CDKN2A* probe shows homozygous deletion of this locus in 88% MPM, but not in RMH. Somatic mutations of the *BRCA1-associated protein 1* (*BAP1*) gene encoding a nuclear deubiquitinase involved in cell growth inhibition are found in 63.6% sporadic MPM.

We examined BAP1 expression by immunocytochemistry (ICC)/immunohistochemistry (IHC) and homozygous deletion of the *CDKN2A* gene by FISH analysis in pleural effusion cytological specimens and pleural tissue biopsy specimens obtained from patients with radiological T0-MPM. We found the loss of BAP1 nuclear staining and/or homozygous deletion of the *CDKN2A* gene in the cytological and biopsy specimens. However, considering that pleural tissue biopsy specimens from radiological T0-MPM may not always contain representative parts of the disease, it is recommended to examine BAP1 expression by ICC and homozygous deletion of the *CDKN2A* gene by FISH analysis using pleural effusion cytological specimens to identify patients with early mesothelioma.

SY11-5

BAP1 Immunostaining and p16 FISH Results in Combination Provide Higher Confidence in Malignant Pleural Mesothelioma Diagnosis

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Objectives: Differentiating malignant pleural mesothelioma (MPM) from reactive mesothelial hyperplasia (RMH) in tissue samples or reactive mesothelial cells (RMC) on pleural effusion smears remains problematic. In this setting, homozygous deletion (HD) of *p16*^{INK4A} (*p16*; detected using fluorescence in situ hybridization (FISH)) and loss of BAP1 protein expression (detected using immunohistochemistry (IHC) or immunocytochemistry (ICC)) are reliable markers for MPM. Use of these assays in combination will likely increase diagnostic accuracy. We investigated the sensitivity and specificity of a combination of BAP1 IHC and *p16* FISH for the differentiation of MPM versus RMH, and also examined cytologic characteristics of *p16* HD or BAP1 loss positive mesothelioma cells.

Methods: BAP1 IHC and *p16* FISH were performed in 51 epithelioid MPM and 20 RMH. We calculated their sensitivities and specificities when the two assays were performed alone or in combination for differentiating MPM from RMH. Morphological characteristics of *p16* HD or BAP1 loss positive cells were also examined using a combination of a virtual microscope system and FISH or ICC.

Results: IHC revealed that loss of expression of BAP1 occurred in 60.8% of MPM. *p16* HD was detected in 60.8% of MPM when FISH was used. The loss of BAP1 using IHC-*p16* HD using FISH was revealed in 84.3% of MPM. This combination had specificity values of 100%. *p16* HD positive MPM cells on pleural effusion smears exhibited significantly more frequent cell-in-cell engulfment, multinucleation (more than two nuclei), and larger multicellular clusters composed of more than 10 cells than *p16* HD negative RMCs. BAP1 loss-positive cells also showed similar morphological characteristics.

Conclusion: A combination of BAP1 IHC and *p16* FISH is the most reliable ancillary tool for differentiating MPM from RMH, and *p16* HD or BAP1 loss positive cells likely show the same morphological characteristics.

Symposium 12

The Bethesda System for Reporting Thyroid Cytopathology: Past, Present, Future

Chairs:
Syed Z. Ali (The Johns Hopkins Hospital, United States)
Philippe Vielh (Cytopathology, Laboratoire National de Santé, Luxembourg)
Leaders:
Diana Rossi (Department of Pathology, Catholic University, Italy)
Marc Pusztaszeri (Department of Pathology, Geneva University Hospital, Switzerland)
William Faquin (Massachusetts General Hospital and Harvard Medical School, United States)
Panel Members:
Justin A. Bishop (Department of Pahology, The Johns Hopkins Hospital, United States), *Ritu Nayar* (Northwestern University, Feinberg School of Medicine/Cytopathology Division and Cytopathology Fellowship Program, Northwestern Memorial Hospital), *Guido Fadda* (Division of Anatomic Pathology and Histology, Catholic University—Foundation 'Agostino Gemelli' Hospital, Italy), *Massimo Bongiovanni* (Institute of Pathology, Lausanne University, Switzerland), *Fernando Schmitt* (Laboratoire National De Santé, Luxembourg), *Beatrix Cochand-Priollet* (Department of Pahology-Cochin Hospital-University Paris Center, France), *Manon Auger* (Department of Pathlogy, McGill University, Canada), *Soonwon Hong* (Department of Pathology, Gangnam Severance Hospital Yonsei University, College of Medicine, Korea), *Mitsuyoshi Hirokawa* (Department of Diagnostic Cytology and Pathology, Kuma Hospital, Japan), *Ashish Chandra* (Guy's and St. Thomas' Hospitals, United Kingdom)

The Bethesda System for Reporting Thyroid Cytology (TBSRTC) was proposed in 2007 at the National Cancer Institute Thyroid Fine Needle Aspiration State of the Art and Science Conference held in Bethesda, Maryland. The aim of this gathering was to provide a road map and discuss in an open forum the inconsistencies and limitations of the available diagnostic terminology at that time for thyroid fine-needle aspiration cytology specimens. The TBSRTC consists of 6 diagnostic categories, each associated with an implied risk of malignancy that translates directly into a clinical management algorithm. The testament of TBSRTC success is its widespread acceptance, development of similar tiered classification schemes around the globe and inclusion in clinical guidelines put forth by the American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE).

Since the publication of the TBSRTC cytology Atlas in January 2010, considerable experience has been gained regarding its application in in cytology practice, clinical impact, and limitations.

In conjunction with the International Academy of Cytology (IAC), an international panel comprising of sixteen cytopathologists and an endocrinologist with special interest in thyroid cytology, including several coauthors of the 2010 TBSRTC Atlas, was created to: 1) analyze the current worldwide impact of TBSRTC, 2) report on the current state of TBSRTC based upon a review of the published literature, and 3) provide possible recommendations

for a future update of TBSRTC. The results of this undertaking will be discussed at a dedicated ICC Symposium, scheduled in Yokohama, Japan, on Monday May 30th (16:15–18:45).

This Symposium will focus on each of the six diagnostic categories of TBSRTC in light of the revised 2015 ATA guidelines, the new 'NIFTP' proposal, and the use of adjunct molecular testing. The Symposium will provide an open forum for audience participation, to address the present and future of TBSRTC.

Disclosure of Interest: None declared.

SY13-1

Cytology and Histology of Lymphoadenitis Representing Epithelioid Cell Response

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Epithelioid cells have oval or elongated nuclei, with larger nuclei than ordinary histiocytes and abundant pale cytoplasm. Epithelioid cell reactions are divided into two patterns; i.e. (i) epithelioid cell singly and in clusters are scattered throughout the cortex and paracortex of the lymph node (ii) Epithelioid cells form round, organized granulomas. Epithelioid granulomatous response classified into two histological subtypes, such as monocytoid B-cell (MBC) positive-granuloma and MBC negative granuloma. The former included cat scratch disease, lymphogranuloma venereum and tularemia lymphadenitis. The latter included sarcoidiosis, tuberculous lymphadentits and yersinia lymphadenitis. This paper describes initially, cytopathological findings of MBC and toxoplasmic lymphadenitis. Secondary, cytopathological findings of cat scratch disease and yersinia lymphadenitis.

Foci of MBCs are located at subcapsular- and medullary-sinuses, and interfollicular areas. Cytologically, two types of MBCs have been delineated as previously reported; (i) The common type of MBC is composed of medium-sized cells with irregular or bean-shaped nuclei and 1–3 inconspicuous nucleoli; (ii) the large cell type is composed of largely transformed MBCs with large round nuclei with vesicular chromatin and 1–3 centrally or peripherally located basophilic, medium-sized nucleoli. Both types of MBC show abundant pale to slightly basophilic cytoplasm and visible cell borders. Both types of MBCs are mixed with varying numbers of neutrophils, small lymphocytes, plasma cells and histiocytes with or without epithelioid cell features.

Characteristic histological triad of toxoplasmic lymphadentis are reactive germinal centers, foci of MBCs and clusters of epithelioid cells throughout the cortex and paracortex. Using fine needle aspiration cytology, microgranulomas composed of a few-pale staining epithelioid cells accompanied by small lymphocytes are quite characteristic.

Cat scratch or bite introduces the bacteria at the site of infection. Two histological stages have been delineated. In early lesions, microabscesses are surrounded by a large collection of MBCs. Numerous histiocytes with or without epithelioid cell features, neutropils, small lymphocytes and scattered plasma cells were intermingled with collection of MBCs. Late lesions contain various numbers of polymorphonuclear leukocytes and deposits of fibrin in necrotic centers surrounded by a thick zone of palisading epithelioid histiocytes. Suppurative granulomas do not contain any or only a few MBCs.

In yersinia lymphadenitis.epitheliod cell granulomas (EPGs) are usually located in the germinal centers. Histological changes include EPGs in various stages of development, with central necrosis associated with neutrophils. EPGs do not contain any MBCs.

Disclosure of Interest: None declared.

SY13-2

Diagnostic Pitfall in Lymph Node Aspiration Cytology

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Fine needle aspiration (FNA) cytology is a simple and useful tool to screen malignant lymphoma involving the lymph node, however may give rise to false negative or false positive results especially in low grade lymphoma. Additional studies including flow cytometric immunophenotypic analysis and gene rearrangement studies may be useful to improve diagnostic accuracy of fine needle aspiration cytology. However such adjunct studies cause increase of medical cost, so usually not been performed in the routine FNA diagnosis of lymph node. Nowadays, immunophenotypic studies as well as genetic studies become more important in the diagnosis of malignant lymphoma, therefore FNA diagnosis depending on the cytologic findings only has a limitation in the diagnostic accuracy in low grade lymphoma including chronic lymphocytic leukemia, marginal zone lymphoma, and follicular lymphoma. In this symposium I would like to share my experience on low grade lymphomas which were misinterpreted in initial fine needle aspiration cytology. In addition, the cases examined by endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) will be discussed. EBUS-TBNA has recently emerged as a minimally invasive technique for evaluation of mediastinal lymph node. In the cases of carcinoma, the usefulness of EBUS-TBNA for evaluating hilar, mediastinal and central parenchymal lesions has been well established. The utility of EBUS-TBNA is now expanding to cover malignant lymphoma and its value for initial diagnosis of lymphoma is under evaluation. A few recent articles have discussed improved accuracy of the diagnosis of lymphoma in EBUS-TBNA through the addition of ancillary techniques, such as immunophenotyping and molecular techniques, however, in routine practice false negative diagnosis may be common especially when the samples are handled by pathologists who have no great concern about lymphoproliferative disease. In this symposium, I would present misdiagnosed cases at initial evaluation and discuss diagnostic pitfall in EBUS-TBNA.

Disclosure of Interest: None declared.

SY13-3

Effusion Lymphoma

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Effusion lymphoma is defined as lymphoma cells in the effusion fluid within the body cavities including pericardial effusion, pleural effusion, and ascites. Malignant lymphomas arising from nodal or extranodal sites may secondarily involve body cavies causing secondary effusion lymphoma. One the other hand, primary effusion lymphoma (PEL) is defined in the 2008 World Health Organization (WHO) classification as large B-cell neoplasm presenting as serous effusions without detectable tumor masses. In the WHO scheme, PEL is universally associated with human herpesvirus 8 (HHV8), also known as Kaposi sarcoma herpesvirus (KSHV), and most often occurs in the setting of immunodeficiency. The majority of the patients are young or middle-aged homosexual or bisexual males with human immunodeficiency virus (HIV) infection and severe immunodeficiency. In addition to HHV8, most of the neoplasms are co-infected with Epstein-Barr virus (EBV). Immunophenotypically, these neoplasms usually lack pan-B-cell markers such as CD19, CD20 and CD79a, but express, instead, CD38, CD138 and EMA. However, in East Asia including Taiwan, a significant proportion of the patients with PEL are old patients without HIV infection and their neoplasms are not associated HHV8 or EBV. Immunophenotypically, they are distinct from 'classical' PEL by the expression of pan-B-cell markers but not CD138. These neoplasms are referred to as 'effusion based lymphoma' in the WHO classification. Some of these patients are associated with liver disease and ascites. The clinicopathological, immunophenotypical and genetic features on common-lymphoma associated chromosomal translocation of PEL vs. effusion-based lymphoma will be illustrated and discussed.

Disclosure of Interest: None declared.

SY13-4

Fine Needle Aspiration Cytology of Unusual Benign Lymphadenopathies Mimicking Lymphoma and Lymphoproliferative Disorders

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Background: Rare and unusual causes of lymphadenopathy presenting like lymphoma include Castleman's disease, Rosai Dorfman disease, Kimura's disease and Kikuchi's lymphadenitis. Cytology of these is sparsely described in the literature.

Methods: All cases of the above diseases diagnosed on histopathology in our hospital for which corresponding fine needle aspiration cytology was available were retrieved and re-evaluated.

Results: There were 12 cases of Castleman's disease which showed cellular lymphoid aspirates with prominence of vascular structures. Epithelioid endothelial cells showed nuclear atypia. None had been diagnosed on cytology. 2/12 cases had inconclusive diagnosis with suspicion of Hodgkin's lymphoma while one case had a positive diagnosis for poorly differentiated carcinoma in an intra-abdominal aspirate. There were 22 cases of Rosai Dorfman's disease, all of which were correctly diagnosed on aspirates with presence of abundant emperipolesis. There were 6 cases of Kikuchi's disease which on aspiration cytology showed crescentic histiocytes with engulfed apoptotic bodies. Two were diagnosed on cytology. There was one case of Kimura's disease in which the aspirate showed presence of germinal center cells, polykaryocytes and many eosinophils.

Conclusion: Benign conditions mimicking lymphoma on clinical presentation can be picked up on fine needle aspiration cytology. Many unusual features are seen on cytology of these conditions, which will be illustrated.

Disclosure of Interest: None declared.

SY13-5

Utility of Liquid-Based Cytology for Diagnosis of Malignant Lymphoma

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Objective: To evaluate utility of liquid-based cytology (LBC) for diagnosis of malignant lymphoma, we compared the cytological features of LBC and conventional preparations.

Study Design: We examined total 80 cases; 5 cases of reactive follicular hyperplasia (RFH), 55 cases of B-cell lymphoma [including 18 follicular lymphoma Grade 1–2 (FL G1–2), 30 diffuse large B-cell lymphoma (DLBCL)], 10 cases of T-cell lymphoma and 10 cases of Classical Hodgkin lymphoma. Cytological specimens from lymph nodes were collected using cytobrush for ThinPrep® method and imprint for conventional method simultaneously. In RFH and FL G1–2, proportions of small cleaved cells, named club cells, was measured using both preparations.

Results and Discussion: To compare to conventional preparation, LBC had clearer background and emphasized nuclear irregularity. In FL G1–2, the proportion of club cells in LBC was higher than that in conventional preparations (19.0% vs. 16.7%, $p < 0.001$). In RFH, the difference was not observed (4.3% vs. 4.0%, $p = 0.601$). These indicated that it may be easy to detect cellular atypia of the lymphoma cells in LBC. We also succeeded in immunocytochemistry (ICC) and fluorescence in situ hybridization (FISH) in LBC.

Conclusion: LBC technique had advantage to evaluate lymphoma cells, especially low grade lymphoma. LBC technique is a useful tool for diagnosis of malignant lymphoma.

Disclosure of Interest: None declared.

SY14-1

Advantages of Digital Image in Cytology

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In association with the recent advance of digital technology, digital pathology including telecytology and digital cytology are getting popular. However, diagnostic pathologists used to estimate digital pathology assuming the superiority of conventional microscopic images. Except for many benefits of image database application, digital images have lots of advantages in the daily cytology diagnosis situation. The essence of digital is numerical conversion. Numerical conversion hold lots of possibilities to help cytology diagnosis. Thinking the fully digitalized pathology laboratory, we assume that all the slides were scanned at the beginning, and the cytology images displayed on the monitor is ready to use lots of utilities. There are many morphological values possibly useful for cytological diagnosis, like 1) nuclear long span, 2) nuclear area, 3) nuclear contour length, 4) nuclear gray scale value, 5) nucleolar area, 6) RGB value of nucleolus, 7) RGB value of cytoplasm, etc. There already exists many utilities for digital images to help diagnosis. Most of them are trying to evaluate whole cells in the field at once, and try to provide conclusive value. This induces many issues like troubles of target cell selection, difficult threshold level to outline the region of interest, etc. On the other hand, we pathologists and cytotechnologists can select target cell by just one click of mouse button on the screen. If we can get above values of one specific atypical cell, we can get lots of help for diagnosis. For example, we can say that this atypical cell have 10 times wider nuclear area than normal cells instead of 'enlarged nuclei' by conventional microscopic observation. The cytoplasm of squamous cell carcinoma is known to show orange color on Papanicolaou stain, but conventional microscopic observation could not provide the threshold RGB value between the orange of cancer cells and the red of normal keratotic cells. We also describe the nuclear chromatin density by RGB value instead of 'increased nuclear chromatin'. Describing these so-called 'atypical' features by numeric value we may increase the accuracy and the reproducibility of cytological diagnosis that conventional microscopy could not provide.

Disclosure of Interest: None declared.

SY14-2

Tools Using in Telecytology and Future Challenges

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Telecytology, TC began sometime after a birth of telepathology, but its extent has been limited. Through reviewing the history of TC in Japan, we will make a survey of the tools using in TC and discuss the requirements for daily diagnosis, consultation and quality assessment.

The authors started the following trial in 1997: a cytotechnologist captured the still images of interesting cells by digital camera, and transferred them to the ftp server via Internet. Dialogue between cytopathologist and cytotechnologist was carried out via e-mail (Still image TC). This method worked till 2011 in Hokkaido. A few followers of still image TC might appear, however, the big flow was not created. Why? We often heard the claim that still image is only a little cut out from the vast microscopic field and also never contains the three-dimensional morphological information of cells.

In 2000s, whole slide image, WSI systems have been developed and considerable WSI scanners were installed at many hospitals in Japan. Cytologists, who thought of the applications for cytology, soon took action to perform the project for external quality assessment and cytology seminars. Though WSI can show the background of the interesting cells in some extent, the focus of cellular image is not enough or obviously inferior to that of still image in many instances. Stacked WSI is also utilized, but the observer's complaint never cool down.

Meanwhile, the authors carried out the intraoperative histological diagnosis through remote sharing of computer desktop containing a video window delivering microscopic field driven by a medical technologist, and expanded this system to TC in 2010 (Desktop video sharing). Currently we have made diagnoses of about 800 cases per a year. The system is similar to the consultation under a discussion microscope, and is built out very easily. However, it has a problem that both cytopathologist and cytotechnologist are constrained by time and location.

In 2009, we proposed Z-axis video for cytology, Zavic. Microscopic field with change in focus is recorded on video and three-dimensional morphological information of cells is clipped from a cytology glass slide. A cytologist can virtually experience a 'focusing through observation' by looking at Zavic. It is very useful for case presentation in cytology seminars, external quality assessment and consultation. Many cytologists give their approvals to Zavic. However, it takes some knowledge about information technology to make a Zavic. Development of the system to make it easily at low price has been expected.

Panoptiq™ is a newcomer in the field of WSI system, which has two unique technical aspects; 1) manual operation like creating a panoramic photo by smartphone, 2) combining WSI with Zavic. It may grow to an optimum system for TC, if some issues are improved.

Disclosure of Interest: None declared.

SY14-3

Present and Future of Telepathology and Telecytology: Focusing on Chinese Experiences

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Background: In recent years, telepathology and tele/digital cytology have played an increasingly important role in pathology consultation and in cytology primary diagnosis. In 2011, China implemented a nationwide telepathology network. By the end of

June 2015, a total of 62,895 pathology cases were submitted to the Chinese central telepathology platform for diagnosis. However, the result of telecytology has not been reported.

Methods: All available pathology cases were extracted from the data base of the Chinese central telepathology platform. The results of telecytology were analyzed and compared with result of telepathology cases. In addition, the current practice in digital cytology in China was also reviewed from Chinese publications, personal observations and communications.

Results: By the end of June 2015, 181 hospitals were connected to the Chinese central telepathology platform and those hospitals submitted a total of 62,895 cases. However, only 48 hospitals submitted 750 cytology cases during this period. Telecytology accounted for only 1.2% of the total telepathology cases. Among the 750 cases, 488 cases (65.1%) were cervical cytology, 262 (34.9%) cases were non-gynecologic cytology. Lung cancer related cytology specimens, such as effusion cytology (177 cases), bronchial brushing and washing (14 cases), lung fine needle aspirate (10 cases) and sputum (7 cases) accounted for 79.1% of non-gynecologic cytology specimens, while head and neck specimens including thyroid fine needle aspiration cytology accounted for 18.7% of non-gynecologic cytology specimens. The agreement rates between submitting pathologists and expert pathologists were 82.38% for cervical Pap cytology cases and 67.56% for non-gynecologic cytology cases.

Conclusion: Although cytology specimens especially cervical Pap cytology specimen contribute to a high proportion of pathology case number, it accounts for disproportionally low percent of telepathology cases in China. A great effort needs be made to change Chinese pathologists's perception for telecytology. Future technology development in digital pathology and in artificial intelligence could eliminate obstacle in adoption of telecytology and contribute to rapid development in digital cytology including telecytology.

Disclosure of Interest: None declared.

SY15-1

Double Immunocytochemical Staining of p63 and CK14 May Be Useful for the Differential Diagnosis of Benign Lesions and DCIS in Fine Needle Aspiration

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The differential diagnosis of benign lesions and ductal carcinoma in situ (DCIS) using fine-needle aspiration biopsy (FNAC) is problematic, because it is difficult to differentiate between the large epithelial clusters associated with benign lesions and those associated with DCIS. Thirty patients who had been diagnosed as having an uncertain malignant potential (indeterminate) for breast cancer on the basis of FNAC findings were selected randomly. The cover glasses of glass slides of specimens stained with the Papanicolaou stain were peeled off, and the specimens were restained with double p63 and CK14 immunocytochemical staining. Benign lesions with ductal hyperplasia of the breast is CK14 immunopositive with the so-called 'mosaic pattern' of immunostaining, whereas malignant cell nests are CK14 immunonegative. And also, the existence of numerous myoepithelial cells which are p63 immunopositive indicates a benign finding in FNAC of the breast. The double immunocytochemical staining of p63 and CK14 was useful for the differential diagnosis of benign lesions and DCIS in FNAC of the breast.

Disclosure of Interest: None declared.

SY15-2

Spindle Cell Lesions – Step by Step Approach in Breast FNA

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Spindle cell lesion of the breast is rare, accounting less than 1% of FNA cytologic specimens. Various lesions of diverse pathology, both malignant and benign, are included in this category. The cytological diagnosis of this kind of lesions is challenging and difficult, because morphological similarities, cytological atypia and mitoses can be seen in both malignant and benign lesions. Thereafter, step by step or algorithmic approach is recommended.

The following parameters are suggested when evaluating a spindle cell lesion in breast FNA: 1) cellular components (spindle cell only or presence of epithelial component; heterologous element; keratinized squamous cells), 2) presence and the degree of atypia, 3) growth pattern, 4) mitotic activity, and 5) clinical and radiological features.

Several examples are demonstrated by such step by step approach: 1) Mixed spindle cell and epithelial proliferations, 2) Pure spindle cell proliferations (bland cytomorphology vs. atypical/malignant cytomorphology), 3) Heterologous element.

Spindle cell lesions of the breast represent a diagnostic challenging subset of diseases, especially in FNA specimens. Step by step meticulous cytomorphology evaluation and clinico-radiologic correlation are very helpful in routine practice.

Disclosure of Interest: None declared.

SY15-4

Atypical Aspirates in Breast Cytology

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Fine needle aspiration cytology (FNAC) is a widely used and important diagnostic modality for breast lesions. The accuracy of FNAC in diagnosing palpable lesions is very high, and it attains a

positive predictive value of around 95–100%. Using a probabilistic approach, FNAC of the breast can be divided into 5 diagnostic categories, namely benign, atypical/indeterminate, suspicious, malignant or unsatisfactory. Among these groups, the atypical category is probably the most controversial, and probably also causes more management and follow up issues.

In the literature, about 30–45% of atypical FNAC turned out to be malignant, and 55–70% turned out to be benign at histologic biopsy or excision. As an atypical FNAC does represent significant cancer risk, all the cases are usually diligently followed, and they usually require histologic assessment, either in forms of a core needle biopsy, mammotome biopsy or excisional biopsy. As these histologic methods are all very effort intensive, it is desirable if one can predict more accurately which atypical breast FNAC would turn out to be malignant on excision, and which not. Cytologic parameters including smears cellularity, percentage of single epithelial cells, epithelial nuclear atypia, bipolar nuclei, background necrosis or histiocytes are some of the commonly investigated cytologic features to predict malignancy. These features will be discussed in greater details.

Disclosure of Interest: None declared.

SY15-5

Breast Marker Antibody Cocktail Immunocytochemistry for Fine Needle Aspiration of the Breast

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Objective: The Breast Marker Cocktail comprises five antibodies recognizing p63, and cytokeratins (CKs) 7, 18, 5, and 14. Immunohistochemistry using this cocktail is useful for diagnosing proliferative intraductal breast lesions. However, immunocytochemistry using the cocktail has not been reported. The aim of study was to know if the immunocytochemistry is useful for the differential diagnosis of breast fine needle aspiration (FNA) cytology.

Materials and Methods: We report 139 cases of mammary samples collected by FNA for which histological diagnoses were available. After cell transfer, immunocytochemistry was performed using the cocktail, and clusters of cells were classified. A cluster with no or limited CK5/14 expression (< 20% of cells) was classified as a monotonous cluster. One with more than 20% of cells showing CK5/14 expression was defined as a mosaic cluster. When at least one p63-positive cell was present, we defined it as a cluster with p63. We also evaluated background p63-positive myoepithelial cell densities.

Results: The histological diagnoses were as follows: fibroadenoma, six cases (5.6%); intraductal papilloma, four cases (3.7%); mucocele-like tumor, one case (0.9%); phyllodes tumors, two cases (1.9%, one benign and one borderline malignancy); atypical ductal hyperplasia, three cases (2.8%); ductal carcinoma in situ (DCIS), 12 cases (11.2%); lobular carcinoma in situ (LCIS), one case (0.9%); invasive carcinoma of no special type, 70 cases (65.4%); mucinous carcinoma, four cases (3.7%); invasive lobular carcinoma, two cases (1.9%); mixed ductal and lobular carcinoma, one case (0.9%); tubular carcinoma, one case (0.9%). The diagnostic sensitivity and specificity for carcinomas were 97.8% (89/91) and 91.7% (11/12), respectively, using the criterion of two or more monotonous clusters lacking p63. Two false negative cases were triple negative cancers; One false positive was an apocrine papilloma. The numbers of monotonous clusters with p63 differed significantly between benign lesions, DCIS/LCIS and invasive carcinomas ($P < 0.001$). The background myoepithelial cell density was significantly higher in fibroepithelial tumours than in other lesions ($P < 0.001$).

Conclusion: Immunocytochemistry using this antibody cocktail showed good sensitivity and specificity for diagnosing breast cancers. Thus, this method is useful for mammary cytology using FNA.

Disclosure of Interest: None declared.

SY16-2

Classification of Thyroid Follicular Cell Tumors – The Day a Cancer Become a Benign Borderline Tumor

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Objectives: In 2009, we proposed a new histologic classification of thyroid tumors which was modified in 2012. In this classification system, we created a new diagnostic category of borderline tumors, between benign and malignant tumors, which had not been established in the 2004 edition of the WHO classification system of thyroid tumors. We proposed borderline tumors are theoretical precursor lesions of thyroid carcinomas based on the multistep carcinogenesis theory, although this knowledge has not been established yet in any textbooks.

Materials and Methods: This category in our classification system includes some tumors formerly termed as carcinomas: 1) non-invasive encapsulated papillary carcinoma (both conventional and follicular variant), 2) well differentiated tumor of uncertain malignant potential (WDT-UMP), 3) capsular invasion-only follicular carcinoma, 4) follicular tumor of uncertain malignant potential (FT-UMP), and 5) low-risk papillary microcarcinoma.

Results: Some of these, such as non-invasive encapsulated papillary carcinoma (only the follicular variant) and the WDTUMP,

were proposed to be renamed as NIFTP (non-invasive follicular neoplasm with papillary-like nuclear features) by Nikiforov et al. in 2015, and papillary microcarcinoma had already been renamed as papillary microtumor by Rosai et al. in 2003, due to the indolent nature of the tumor. We expect further discussion on the other low-risk and indolent thyroid tumors and their placement in the borderline malignancy or precursor lesion category in the future.

These tumors are generally curable with simple removal, and do not require more aggressive treatments, such as total thyroidectomy with radioactive iodine treatment.

Conclusion: We are soon leaving a historical period of thyroid pathology, when pathologists have had only two diagnostic choices for thyroid tumors (benign and malignant), and are entering a new era with three choices (benign, borderline, and malignant), similar to classification systems utilized in other organ systems. We believe it is important to reduce overdiagnosis and overtreatment of patients with such indolent tumors.

Disclosure of Interest: None declared.

SY16-3

Impact of Noninvasive Follicular Variant of Papillary Thyroid Carcinoma on Malignancy Risk for Fine-Needle Aspiration of Thyroid Lesions

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Fine-needle aspiration (FNA) is a widely accepted, cost-effective, and safe method for triaging patients with thyroid nodules. The Bethesda System for Reporting Thyroid Cytopathology has been widely adopted for reporting the results of thyroid FNAs. The 6 diagnostic categories of the Bethesda System are (i) nondiagnostic/unsatisfactory; (ii) benign; (iii) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS); (iv) follicular neoplasm/suspicious for follicular neoplasm (FN/SFN), a category that also encompasses the diagnosis of Hürthle cell neoplasm/suspicious for Hürthle cell neoplasm; (v) suspicious for malignancy (SUSP), and (vi) malignant. The Bethesda System provides an estimation of cancer risk within each category. The estimated rates of malignancy for each diagnostic category are as follows: non-diagnostic, 1–4%; benign, 0–3%; AUS/FLUS, 5–15%; FN/SFN, 15–30%; SUSP, 60–75%; and malignant, 97–99%. Based on the meta-analysis of eight studies reported by Bongiovanni *et al*, the actual rates of malignancy in nodules surgically excised are as follows: non-diagnostic, 20% (9–32%); benign, 2.5% (1–10%); AUS/FLUS, 14% (6–48%); FN/SFN, 25% (14.34%); SUSP, 70 (53–97%); and malignant, 99% (94–100%).

Follicular variant of papillary thyroid carcinoma (FVPTC) is the most common type of malignant tumor among thyroid nodules with a preoperative diagnosis of AUS/FLUS or FN/SFN. Noninvasive FVPTCs have virtually no metastatic potential or risk of recurrence and are more akin to follicular adenomas. If noninvasive FVPTC were no longer considered malignancy, the rate of malignancy for preoperative diagnosis of AUS/FLUS, FN/SFN, and SUSP would be significantly decreased. A change in terminology will affect the cancer rates of these categories. I will present recent data about noninvasive FVPTC and discuss its impact on malignancy risk for fine-needle aspiration of thyroid lesions.

Disclosure of Interest: None declared.

SY16-4

Challenging Pathological Features of the Fine Needle Aspiration in Thyroid: A Review by Cytological Histological Correlations

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Objectives: Fine needle aspiration (FNA) is essential for the management of thyroid lesions in particular in the evaluation of thyroid nodules and detection of lymph node metastases in thyroid malignancies. The objectives are to review the cytological and histological features of the thyroid nodules based on the Bethesda System for Reporting Thyroid Cytopathology (BSRTC).

Materials and Methods: Consecutive cases of thyroid nodules having both cytology and biopsy were retrieved. A presentation of our experience in the pathology of thyroid nodules by studying the correlations of the results of FNA using BSRTC and biopsy was done.

Results: BSRTC are useful in predicting the histology in majority of the cases. A few cases with cytology and histological features challenging to the pathologists were noted. The features will be discussed to enrich our experience in diagnostic thyroid pathology.

Conclusion: Histology and cytology correlations will enrich pathologist experience in diagnostic thyroid pathology.

Disclosure of Interest: None declared.

SY17-1

Clinical Efficacy and Immunogenicity of the Nonavalent HPV Vaccine

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Objectives: An efficacy and immunogenicity study of an investigational 9-valent HPV (6/11/16/18/31/33/45/52/58) (9vHPV) vaccine was conducted in women 16–26 years of age to demonstrate immunological non-inferiority of HPV 6/11/16/18 response and efficacy against HPV 31/33/4/52/58-related persistent infection and disease. The report presents results through end-of-study (i.e. up to month 54).

Methods: 14,204 healthy 16–26 year-old women were enrolled into an international, double-blind efficacy and immunogenicity study of the 9vHPV vaccine. Subjects received 9vHPV vaccine or quadrivalent HPV vaccine (qHPV) as a series of injections at day

1/month 2/month 6. Primary analyses included subjects who were seronegative at day 1 and PCR negative from day 1 through month 7 for the HPV type being analyzed. Gynecological swabs (for HPV DNA testing) and Pap test were performed every 6 months. Subjects with abnormal Pap tests were referred to colposcopy.

Results: Anti-HPV 6/11/16/18 responses generated by 9vHPV vaccine were non-inferior to those generated by qHPV vaccine. Efficacy of 9vHPV vaccine against a composite endpoint of HPV 31/33/45/52/58-related high-grade cervical/vulvar/vaginal disease was 97.4% ([95% CI: 85.0–99.0] 1 case in the 9vHPV vaccine group and 38 cases in the qHPV vaccine group). Efficacy against HPV 31/33/45/52/58-related cervical/vulvar/vaginal disease (any grade) in the PPE was 97.7% (95% CI: 93.3, 99.4). Efficacy against HPV 31/33/45/52/58-related 6-month persistent infection in the PPE was 96.0% (95% CI: 94.6–97.1).

Conclusions: The 9vHPV vaccine was highly efficacious in preventing HPV 31/33/45/52/58-related persistent infection and disease up to month 54. HPV 6/11/16/18 immune responses were non-inferior to that of qHPV vaccine.

Disclosure of Interest: Research funding to the Medical University vienna by Merck. Advisory Board fees and lecture fees by Merck.

SY17-2

Neurological Manifestations Following HPV Vaccine Immunization

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A relatively high incidence of chronic limb pain, frequently complicated by violent, tremulous involuntary movements, has been noted in Japanese girls following human papillomavirus (HPV) vaccination. The average incubation period after the first dose of the vaccine was 5.47 ± 5.00 months. Frequent manifestations included headaches, general fatigue, coldness of the legs, limb pain and weakness. The skin temperature examined in the girls with limb symptoms exhibited a slight decrease in the fingers and a moderate decrease in the toes. Digital plethysmograms revealed a reduced height of the waves, especially in the toes. The limb symptoms of the affected girls were compatible with the diagnostic criteria for complex regional pain syndrome (CRPS). The Schellong test identified a significant number of patients with orthostatic hypotension and a few patients with postural orthostatic tachycardia syndrome. Electron-microscopic examinations of the intradermal nerves showed an abnormal pathology in the unmyelinated fibers in two of the three girls examined. The symptoms observed in this study can be explained by abnormal peripheral sympathetic responses. The most common previous diagnosis in the studied girls was psychosomatic disease. Recently delayed manifestation of cognitive dysfunction in the post-vaccinated girls has been paid much attention: memory loss, difficulty in reading textbooks and/or calculation.

SY17-3

The HPV Vaccination Crisis in Japan

Sharon J.B. Hanley

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Ten years after the HPV vaccines were first licensed, 65 countries have them in their national immunization program (NIP) and the number of low and middle-income countries with national programs is increasing. Countries such as Panama, Bhutan, Malaysia and Rwanda have three dose uptake rates close to or more than 90% and several high-income countries have >80% coverage. Regardless of economic status, for most countries the trend in uptake is upwards. The notable exception, however, is Japan.

Free vaccination against HPV began in December, 2010, for Japanese girls aged 12–16 years and since April, 2013, the vaccine was included in the NIP. However, in June 2013, the Japanese Ministry of Health, Labour and Welfare (MHLW) suspended proactive recommendations for HPV vaccines after unconfirmed reports of adverse events following immunization (AEFI) appeared in the media. Despite the Vaccine Adverse Reactions Review Committee repeatedly concluding no evidence exists to suggest a causal association between the HPV vaccine and the reported AEFI, as well as those purporting the adverse events being unable to demonstrate any consistent, biologically plausible temporal relationship between vaccination and disease, the MHLW has failed to reintroduce proactive recommendations for the vaccine. By not responding rapidly and scientifically, the consequences have been devastating with three dose uptake plummeting from >70% to <1%.

In the 10 years since licensing, over 230 million doses of the HPV vaccine have been used in population-based programs globally and high quality analytical studies on vaccine safety have shown no association between either vaccine and neurological, thromboembolic or autoimmune diseases. A recent study of 70,000 vaccinated and unvaccinated women in Nagoya, Japan also found no association between vaccination and purported AEFI.

Sadly, the MHLW has been hit with a better organized anti-vaccination movement who have gained control of the narrative through offline, online/social media, sensational video clips and highly publicized events. Furthermore, to exacerbate the situation, the MHLW has never refuted or criticized false media reports. No vaccine safety signal has been recorded in Japan. Instead, individuals who have the misfortune to be unwell with rare or difficult to treat disorders have been encouraged by anti-vaccination advocates to blame the HPV vaccine, especially in an unrestrained media environment and with little reassurance and systematic addressing of these events by the government.

Reduced dose schedules, licensing of the nonavalent HPV vaccine and subsidized vaccine introduction through organizations such as GAVI and PAHO means the benefits in cancer protection are being expanded to wider populations. The question that remains is when will Japan be one of them.

Disclosure of Interest: None declared.

SY17-4

Human Papillomavirus Vaccines: Global Versus Japanese Situations

Rintaro Mori

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Globally, cervical cancer, which ranks the 4th cause of female cancer and 2nd most common female cancer in women aged 15 to 44 years in the world (estimations for 2012), is the causes of cancer death in women. Cumulative risks for women ages between 0 and 74 years estimated for 2012 were 1.4%, 1.6% and 0.9% for world, less developed regions and more developed regions, respectively. In particular, the biggest burden were in Sub-Sahara Africa and Melanesia. Combination of HPV vaccines and screening programmes may reduce the risk of cervical cancer, and there are a number of systematic reviews and meta-analyses, as well as economic evaluations, of the HPV vaccines conducted to assess effectiveness of the vaccines. The results showed that there was significant effect on prevention of HPV infection for up to 5 to 9 years, though effects on preventing cervical and other cancers were calculated as estimates extrapolating evidence from decreased incidence of pre-cancer changed in cervices, due to the fact that the product was developed relatively new. While screening programme in high income settings had been promoted, HPV vaccine was first licenced in 2006, and over 200 million doses have been distributed ever. The World Health Organization, as well as the majority of high income countries, taking limitations of the available evidence into account, recommends HPV vaccines as a part of national immunization programmes. To date, 58 countries including low/middle countries had introduced HPV vaccine as parts of their national immunization programmes. HPV vaccine was first licensed in 2009 in Japan, and it fully became a part of national immunization programmes in April, 2013. However, chronic pain and other symptoms were reported in some vaccine recipients, and this led to suspension of the proactive recommendation for routine use of HPV vaccine in the national immunization programme in June, 2013. Table 1 shown below are HPV vaccination coverage reported in HPV information centre.

The data clearly shows unique situation in Japan, where the coverage reported is 0.6%, while the great majority of countries where national program exist have over 50%. The talk will attempt to address why the changes happened and considerations around the difference.

Disclosure of Interest: None declared.

SY18-1

Introductions and USA

Ritu Nayar

Northwestern University, Feinberg School of Medicine/ Cytopathology Division and Cytopathology Fellowship Program, Northwestern Memorial Hospital, United States

This symposium will provide a snapshot view of the state of cervical cancer prevention – the good and not so good news. In the interest of time the regions covered will be USA, UK, Australia, and Europe. The speakers will discuss prevention – success of program(s), current state of screening and vaccination, and obstacles and concerns. A brief update of the ESTAMPA multicentric study of cervical cancer screening and triage with HPV test being

Table 1. Full course HPV vaccine coverage by countries (shown only those reported) (Modified from Bruni 2015) (for Abstract SY17-4)

Country	Coverage	Year reported	Country	Coverage	Year reported
Argentina	50%	2013	Malaysia	87%	2011
Australia	73.1%	2014	Mexico	67%	2010
Belgium	29–82%	2012	Netherlands	61%	2014
Bhutan	92%	2010	New Zealand	56%	2014
Canada	60–85%	2013	Norway	79%	2014
Colombia	87%	2013	Panama	67%	2010
Denmark	82%	2015	Portugal	87%	2015
Finland	68%	2015	Romania	<5%	?
France	25.0–29.3%	2012	Rwanda	99%	2013
Germany	40%	2012	Slovenia	49%	2012
Greece	5–27%	2011	South Africa	87%	2014, First Dose
Iceland	88%	2012	Spain	73%	2014
Ireland	84.9%	2014	Sweden	80%	2014
Italy	71%	2014	Switzerland	51%	2013
Japan	**0.6%**	**2014 in Sapporo after suspension**	United Arab Emirates	59%	2011
Latvia	60.6%	2011	United Kingdom	86%	2014
Luxembourg	17%	2009	United States of America	39.7%	2014
Macedonia, TFYR	65%	2012			

conducted in 10 Latin American countries and the ANCHOR study for anal cancer prevention will also be included. In the last 30 minutes of the session, the speakers will have 5 minutes each to give their prediction on the future of cervical cancer screening and prevention followed by 15 min. of comments and Q/A with the audience.

Disclosure of Interest: None declared.

SY18-2

Cervical Cancer Prevention in the 21st Century – Australia

Annabelle Farnsworth

Douglass Hanly Moir Pathology, Australia

Australia has had an organised cervical screening program since 1991 funded by the Australian Federal Government. It provides money for programs to encourage participation, educational material for clinicians and patients, quality standards for laboratories, and funds for state based registers. The program has been highly successful with a halving of incidence and mortality from cervical cancer during that time. The screening test is currently conventional cytology. Liquid based cytology with automation is used in approximately 25% of cytology samples but this is paid for by the patient and is still read as a split sample. Human papillomavirus (HPV) testing has been used for patient management following treatment of know high grade lesions since 2006.

An Australian Government funded HPV vaccination was introduced in 2007. The quadrivalent vaccine is delivered free of charge to both girls and boys in their first year of high school. Catch up programs were also provided at the outset. The HPV vaccinisation has achieved a70% coverage. Studies have shown a fall in the incidence of the virus types which were included in the quadrivalent vaccine. Current data also shows a fall the rate of high grade cervical disease in the vaccinated cohort.

Because of the success of the vaccination program, Australia is currently in the process of renewing its cervical cancer prevention strategy and is planning to introduce HPV testing as the primary screening method with cytology triage in 2017. The test will be offered every five years to women commencing at age 25.

Disclosure of Interest: None declared.

SY18-4

Current Status and Challenges in Western Europe

Christine Bergeron

Department of Pathology, Laboratoire Cerba, France

Cervical cancer incidence and mortality will be presented in Western Europe. The diversity of screening approaches will be described. The projects and challenges these countries will have to face in the near future will be discussed.

Disclosure of Interest: None declared.

SY18-5

Latin America (ESTAMPA) and Anal (ANCHOR)

Teresa M. Darragh

University of California San Francisco, United States

While much of the developed world has enjoyed remarkable success with cervical cancer screening and the initiation of HPV vaccination, cervical cancer prevention strategies in many countries in the developing world are in their infancy. Preliminary results from IARC's ESTAMPA study of cervical cancer screening and triage with HPV testing that is being conducted in 10 Latin American countries will be presented. ESTAMPA's primary objective is to estimate the performance characteristics (sensitivity, specificity, positive and negative predictive value) of multiple techniques alone or in combination for detection of HSIL on biopsy among HPV positive women, 30–64 years old.

Anal cancer is also an HPV-associated malignancy. The rates of anal cancer in high-risk populations, particularly those with HIV disease, equal or exceed cervical cancer rates in many areas of the world – even in countries with little or no cervical screening. The ANCHOR study for anal cancer prevention will be discussed. ANCHOR is a multicenter trial in the United States; its primary objective is to determine the effectiveness of treating anal high-grade squamous intraepithelial lesions (HSIL) to reduce the incidence of anal cancer in HIV-infected men and women.

Disclosure of Interest: None declared.

Symposium 19

The Paris System for Reporting Urinary Cytology

Chairs: *Eva M. Wojcik* (Loyola University, United States)
Dorothy Rosenthal (Pathology, Johns Hopkins School of Medicine, United States)

Speakers:
Sachiko Minamiguchi (Department of Diagnostic Pathology, Kyoto University Hospital, Japan)
Christopher VandenBussche (Pathology, Johns Hopkins School of Medicine, United States)
Toyonori Tsuzuki (Department of Pathology, Japanese Red Cross Nagoya Daini Hospital, Japan)
Matthew Olson (Pathology, Johns Hopkins University School of Medicine, United States)
Daniel F. Iyama-Kurtycz (Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, United States)

The Paris System for Reporting Urinary Cytology has been developed by members of the American Society of Cytopathology and the International Academy of Cytology to establish a standardized and reproducible reporting system that is based on clinical and pathologic evidence, diagnostic consensus and understanding of the pathogenesis of urothelial carcinoma. The System emphasizes the goal of performing urine examination, i.e., detec-

tion of High Grade Urothelial Carcinoma (HGUC), while minimizing the detection of Low Grade Urothelial Neoplasia (LGUN). In addition, the indeterminate category of Atypical Urothelial Cells (AUC) has been well defined with a goal for this category to become a clinically relevant one. Criteria for each category have been based on clinical outcomes. This workshop will describe the criteria that the Paris Working Group has established for each diagnostic category. Furthermore, risk stratification for developing HGUC for each diagnostic category and, therefore, patient management will be discussed.

The main objectives of this workshop are:

1. Understand the rationale for standardizing urinary cytology reporting based upon strict morphologic criteria.

2. Appreciate clinical management implications of the diagnostic categories.

3. Relate the relative risk of HGUC according to the results of the cytologic interpretation for each diagnostic group.

SY19-1

Japanese versus Paris System for Reporting Urinary Cytology, Differences and Similarities

Sachiko Minamiguchi, Takaki Sakurai, Shinsuke Shibuya, Hiroyuki Shirahase, Hironori Haga

Department of Diagnostic Pathology, Kyoto University Hospital, Japan

The use of various systems for reporting urinary cytology among different pathologists, cytotechnologists, and institutions can lead to confusion in the clinical practice of managing bladder cancer. In recognition of the necessity for a standardized and comprehensive reporting system for urinary cytology, a Japanese working group was inaugurated in 2012, and an international working group was established in Paris in May of 2013 at the 18th International Congress of Cytology. The Japanese working group designed the Japanese reporting system for urine cytology in 2015 with exchange of information with the Paris system (TPS). In 2016, the TPS group published a book titled *The Paris System for Reporting Urinary Cytology*, focusing on the detection of high-grade urothelial carcinoma (HGUC). Both TPS and the Japanese System have the assessment of specimen adequacy and four categories for the assessment of high-grade urothelial carcinoma (HGUC).

The terminologies of four categories in each system are;

The Japanese System: Negative, Atypical, Suspicious, Malignant.

TPS: Negative for HGUC (NHGUC), Atypical urothelial cells (AUC), Suspicious for HGUC (SHGUC), HGUC.

The most important role in urine cytology is the detection of lesions that are difficult to find cystoscopically, and to recognize HGUCs that have a high risk of evolving into life-threatening invasive lesions. Both TPS and the Japanese system emphasize these purposes.

However, there is a difference between the two systems in the definitions of Atypical and Suspicious. In TPS, the criteria for AUCs are relatively strictly defined as non-superficial and non-degenerated urothelial cells with an N/C ratio >0.5, and one of the following features: 1) hyperchromasia; 2) irregular coarse, clumped chromatin; or 3) irregular nuclear membranes. If the N/C ratio is >0.7 and two of the features listed above are present, the diagnosis should be 'Suspicious for HGUC'. In the Japanese system, the 'Atypical', 'Suspicious', and 'Malignant' categories include the possibility of low-grade urothelial carcinoma/neoplasm (LGUN) and other types of neoplasm. TPS focuses more on the detection of HGUC, and recognizes the inability to reliably detect LGUN by routine cytology. The Japanese working group adopted the 'Malignant' category because urine cytology is used not only by urologists, but also by other clinicians who manage non-urothelial cancers with bladder metastasis.

In adopting these new systems, cyto-histological correlation is critical for establishing better clinical guidance for bladder cancer cystoscopy and treatment strategies. From the beginning of 2016, our department has adopted the use of both systems, and I would like to present several cases to discuss in this presentation.

Disclosure of Interest: None declared.

SY19-3

Diagnosing High Grade Urothelial Carcinomas and Other Malignancies

Toyonori Tsuzuki, Shuko Seko

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High grade urothelial carcinoma (HGUC), including invasive urothelial carcinomas and urothelial carcinoma in situ, is the most important disease category in the field of urogenital oncology. Therefore, the Paris System (TPS) has focused on HGUC. Urologists consider urine cytology as a less invasive diagnostic tool for detection of newly developed HGUCs and surveillance of HGUCs after various therapies.

Although urine cytology demonstrates a high specificity, it has a relatively low sensitivity, even for HGUCs. Several cytological features such as high nuclear to cytoplasmic (N/C) ratio, nuclear pleomorphism, nuclear membrane irregularity, hyperchromasia, irregular chromatin pattern, prominent nucleoli, mitosis, apoptosis etc. are well-known diagnostic criteria for HGUC among specialists. However, HGUCs fulfilling all these criteria are relatively rare. Unnecessary criteria could be the major causes for low sensitivity of urine cytology in detecting HGUCs. TPS proposes new diagnostic criteria for HGUCs in order to standardize and improve their detection sensitivity by urine cytology. TPS's survey has revealed that many experts prioritize the following cytological features during diagnoses: high N/C ratio (0.7 or greater), nuclear hypechromasia, irregular nuclear membranes, and coarse chromatin. TPS proposes the aforementioned cytological features as the new diagnostic criteria for HGUCs and requires at least five to ten viable malignant cells to fulfill these criteria before definite diagnosis. We present these diagnostic criteria and demonstrate how to recognize and apply them.

Non-urothelial carcinomas are uncommon, accounting for only less than 5% of bladder cancers. The therapeutic strategies for them and secondary (metastatic) bladder cancer require different

management and interventions from those for urothelial carcinomas. Therefore, considering these diseases as a distinctive entity is beneficial for urologists because it would make it easier to recognize these diseases as different from HGUC. Unfortunately, some cases of non-urothelial carcinomas are diagnosed as 'HGUC' because of their rarity. We present representative cases of non-urothelial carcinomas and our key diagnostic findings.

Disclosure of Interest: None declared.

SY20-1

A Pathologist's View on Cytodiagnosis of MALT Lymphoma – A Brief Introduction to the Symposium

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Cytodiagnosis of small B-cell lymphomas including extranodal marginal zone lymphoma of MALT type – MALT lymphoma – is often challenging and misleading especially in FNA cytology specimens. The tumor cells look similar mutually in cytology, just small and round, and there have been no cytomorphological clues so far to the differential diagnoses of these look-alike lymphomas.

Surgical pathologists usually regard the diagnosis of MALT lymphoma as that of 'exclusion' and often have difficulty in differentiating it from other reactive inflammatory lesions because most MALT lymphoma arises gradually with a background of chronic inflammation including H. pylori-related chronic active gastritis, Hashimoto thyroiditis, Sjögren's syndrome, etc. As described in the current WHO blue book, in addition to the basic morphology, diagnosis of MALT lymphoma including such difficult gray-zone cases often requires multidisciplinary/multiparameter approaches – immunophenotyping by flow cytometry, immunohisto-cytochemistry, Southern blotting, chromosomal-gene analysis, etc.

In general, clinicians and pathologists often pay scant attention to the diagnosis of MALT lymphoma by FNA cytology because they simply believe that MALT lymphoma cannot be diagnosed by only cytology for the reason that cytology fails to evaluate the important histological clues such as lymphoepithelial lesion and follicular colonization, and the valuable multidisciplinary approaches mentioned above are not applicable to the FNA cytology specimens.

Actually, because of a considerable progress of current study on hematolymphoid cytology, FNA cytology of MALT lymphoma, e.g. thyroid, lung, breast and salivary gland, has often become fairly successful in making a proper cytodiagnosis.

In this introductory talk, a brief explanation as to the basic cytomorphological findings of MALT lymphoma including the differential diagnoses of other small B-cell lymphomas and why the cytological diagnosis of MALT lymphoma – a cytological 'sanctuary' of small B-cell lymphomas – is so challenging will be provided.

Disclosure of Interest: None declared.

SY20-2

Cytological Diagnosis of MALT Lymphoma: Diagnostic Criteria and Differential Diagnosis

Andrew S. Field

Notre Dame University Medical School and St, Vincent's Hospital, Australia

Lymphomas arising in mucosa associated lymphoid tissue and marginal zone extranodal lymphoma are uncommon B-cell lymphomas. The FNAB diagnosis of MALT lymphomas can be difficult early in the evolution of the tumor due to the presence of large numbers of reactive germinal centres and the lack of a pathognomonic immunophenotype. An approach to the FNAB diagnosis and the differential diagnosis based on a pattern recognition assessment of the smears integrated with analysis of specific cell types will be presented. The cytological criteria include a heterogeneous population of lymphocytes, centrocytes, monocytoid cells with clear cytoplasm, plasmacytoid cells, plasma cells and occasional larger lymphoid cells. There may be a vague nodularity with residual germinal centres with their dendritic cells and usually only occasional tingible body macrophages. The differential diagnosis will be discussed and includes follicular lymphomas and all the small cell lymphomas including mantle cell, small lymphocytic and lymphoplasmacytic lymphoma. The cytomorphological assessment should be integrated with the flow cytometry and cytogenetic findings.

Disclosure of Interest: None declared.

SY20-3

Cytological Findings of MALT Lymphoma by Means of FNA in Thyroid Gland

Sadayuki Kaba

Gunma Paz College, Japan

Objectives: We examined cytological findings based on Papanicolaou-stained smears for the diagnosis of primary thyroid MALT lymphoma by fine needle aspiration.

Materials and Methods: During the study period of 4 years at Kuma Hospital (Kobe, Japan), a total of 101 cases including 51 MALT lymphomas, 20 Hashimoto's thyroiditis (HT), and 30 DLBCL were cytologically examined. MALT lymphomas were divided into 44 Common-MALT and 7 MALT lymphomas with extreme plasmacytic differentiation (MALT-EPCD).

To prepare smear specimens, aspirate samples obtained by ultrasound-guided thyroid FNAC were plated onto glass slides, covered with another slide, and then smeared by separating the slides vertically. The smears were fixed with Cytrop and then stained by Papanicolaou stain.

In the present study, the discriminant factors and accuracy rates were obtained using JMP8.

Results: 1) Small to medium-sized cells displaying ISN-PN (irregularly-shaped nucleus with prominent nucleolus) findings

were neoplastic cells. 2) In the case of a frequency of plasma cells (PCs) below 15%: the accuracy rate for distinguishing Common-MALT from HT was 97% by ISN-PN cell frequencies more than 20% in combination with the presence of LELCs and MRLCs. 3) The frequency of large-sized cells was less than 15% in Common-MALT, while it was more than 15 in DLBCL. The accuracy rate of large-sized cell frequencies more than 15% being able to distinguish between both lesions was 96%. 4) In the case of a frequency of PCs above 15%: cases with 'sum of PCs plus ISN-PN cells' being above 30% were MALT-EPCD. PCs/CCL-cells were CD20 and CD56 uniformly-negative on the histological specimen. Plasma cells and CCL cells in most of MALT-EPCD cases were predominantly kappa-light chain-positive. 5) MRLCs (mountain range-like clusters) were cell clusters including follicular dendritic cells, tingible body macrophages and ISN-PN cells, which were derived from regions of follicular colonization. MRLCs were detected in 89% of Common-MALT cases. LELCs were cell clusters in which multiple ISN-PN cells were detected among Hurthle cells, which were derived from regions of lymphoepithelial lesions. LELCs were detected in 75% of Common-MALT cases. Cellular clusters seen in HT were including a few small lymphocytes among Hurthle cells. Those clusters were detected in 80% of HT cases.

Conclusion: It is necessary that thyroid common-MALT discriminates from HT, DLBCL and MALT-EPCD. The possibility to discriminate from other lesions by the useful cytological findings of common-MALT based on Pap-stained specimen will be discussed.

Disclosure of Interest: None declared.

SY20-4

Usefulness of Cytology and Molecular Features in the Diagnosis of MALT Lymphomas

Franco Fulciniti[1], Anna Cipolletta Campanile[2], Gabriella Malzone[2], Veronica Sanna[2], Jessica Barizzi[1], Elisabetta Merlo[1], Annarosaria De Chiara[2]*

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Objectives: We searched the database of 2 Institutions for cases with a cytological diagnosis of MALT (mucosa-associated lymphoid tissue)/extranodal Marginal zone lymphoma (MZL) and found 11 confirmed cases in the period Jan 1st, 2010-Dec 1st, 2015. We verified the feasibility of the diagnosis, and its predictive value together with other special techniques.

Material and Methods: The databases of 2 Institutions were searched for cases with a cytological diagnosis of MALT/MZL. 11 such cases were found, concerning 6 F and 5 M patients (age range 30–79, median age 69 years). Cyto-histopathological material and data from ancillary studies, were retrieved and re-examined. The role of supplementary tests, like flow cytometry (FCM), FISH analyses and clonality studies was evaluated.

Results: Sites and diagnostic sample types were: soft tissues: 3 (Fine Needle Aspirations: FNA), superficial lymphnodes: 2 (FNA), mediastinal nodes: 1, (TB-FNA), thyroid: 1 (FNA), pleural effusions: 2, broncho-alveolar lavage (BAL): 1, bronchial brushing: 1. A diagnosis of MALT/MZL was rendered on 2/3 soft tissue masses (1/3 was diagnosed as a G1 follicular lymphoma), on 2/2 pleural effusions, 2/3 lymph nodes (1/3 was diagnosed as a DLBCL), on 1 thyroid lesion and on a bronchial brushing sample. Totally, a diagnosis of MALT/MZL was rendered in 9/11 cases (81.8%). ICC techniques were adopted in 6/11 cases, of which 4/11 (36.3%) were FNAs and 2/11 (18.1%) were pleural fluids. FCM was used alone (5 cases) or in conjuntion to cytogenetic techniques in 3 FNA samples. The API2-MALT1 FISH was used in two cases: a pleural effusion and a bronchial brushing sample and permitted a correct diagnosis in cases with only moderate cellularity. Before the introduction of this test, FISH on cytologic samples had been already used on cytological samples in conjunction to ICC or FCM to exclude follicular B-cell lymphoma, with negative results for the t: (14.18).

Conclusions: The diagnosis of MALT/Marginal zone lymphoma on cytopathologic material is challenging. A correct diagnosis depends on the availability of sufficient, high quality cytologic material. FNA samples taken by trained cytopathologists and fluid specimens usually have a higher cellular content and better material preservation. Rapid on-site examination of FNA samples taken by the cytopathologist permits an initial diagnostic assessment which is frequently useful to prepare dedicated cell samples. The recent introduction of the API2-MALT1 FISH test should still be thoroughly evaluated on cytologic material, but its application on two of our cases has led to a precise diagnosis.

Disclosure of Interest: None declared.

Slide Seminars

SS01-1

Keynote Lecture: General Aspects of Endocervical Adenocarcinoma and Its Related Lesions

Rana S. Hoda

New York Presbyterian Hospital, Weill Cornell Medical College, United States

There has been a 29% increase in incidence in recent years for invasive Endocervical Adenocarcinoma (ECA). In 1970, ECA comprised approximately (~) 12% & squamous cell carcinoma (SqCC) ~ 88%. While in 2000, ECA comprised ~ 29% & SqCC ~ 69%. This increase in ECA may be due to earlier detection by new sampling devices or because of increased application to HPV tests in Pap tests. Gross appearance of ECA is variable and prognosis may be worse than SqCC. Morphologically, ECA can be usual type, Mucinous, minimal deviation adenocarcinoma, villoglandular papillary adenocarcinoma and others (Endometrioid, Clear cell, Serous, Mesonephric).

HPV test plays an important role in detecting endocervical glandular lesions. For a cytological diagnosis of atypical glandular cells (AGC), the sensitivity of HPV for significant lesions is 83% &

specificity of 82%. HPV 16 and 18 most common types. For a cytological diagnosis of glandular neoplasia, the sensitivity for AIS is 86%–100%, sensitivity for ECA is 85%–94% & specificity & negative predictive value of 100% & 97% respectively.

The two liquid-based preparations (LBP) currently in use include, ThinPrep (TP) and SurePath (SP). Morphological appearance of glandular lesions, both endocervical and endometrial, in LBP is comparable to conventional smears, however, key differences exist. The differences are due to different collection and processing techniques.

Disclosure of Interest: None declared.

SS01-2

Session: Slide Seminar on Cytopathology of Cervical Adenocarcinoma

Wanwisa Himakhun

Thammasat University, Thailand

Method of Sample Collection: Conventional Pap smear.

Clinical Summary: A 36 years old women came for infertility consultation. She did not have any history of illness. Her physical examinations showed normal vital sign and unremarkable per vaginal examination. The trans-vaginal sonogram showed one subserous leiomyoma. The laboratory tests for serology were non-reactive for Anti HIV and VDRL and negative for Rubella IgG and HBs Ag. A conventional Pap smear for routine screening was performed on 10th March 2015.

Disclosure of Interest: None declared.

SS01-3

Slide Seminar on Cytopathology of Cervical Adenocarcinoma

John H.F. Smith

Sheffield Teaching Hospitals NHS Foundation Trust, United Kingdom

39 year old woman. Asymptomatic. Routine cervical smear. All previous cervical cytology normal.

SurePath LBC sample.

Disclosure of Interest: Speaker bureau BD Europe and BD Asia-Pacific.

SS01-4

Gastric Type Adenocarcinoma in situ

Tsui-Lien Mao

Department of Pathology, College of Medicine, National Taiwan University Hospital, Taiwan

Case History: A 74-year-old G3P3 woman was found to have 'atypical glands' in her recent routine Pap smear. Tracing back her history, she was asymptomatic, with no bloody or watery vaginal discharge. On pelvic examination, the uterine cervix showed atrophic change. Colposcopy disclosed no acetowhite lesion. Dilation and curettage revealed 'atypical glands'. Hence, she received loop electrosurgical excision procedure (LEEP) subsequently.

Disclosure of Interest: None declared.

SS01-5

Cervical Cancer

Isabel Alvarado-cabrero

Pathology Department, Mexican Oncology Hospital, IMSS, Mexico

A 69-year old woman was admitted for investigation of abdominal pain and disuria.

Past medical history: at the age of 62, she developed hypertension. She had never had gynaecological problems or a Papanicolaou smear. Laboratory results included a normal hematology and biochemistry panels excluding anemia, renal failure or liver disease.

Physical examination revealed no abnormal findings, and gynecological examination showed an exocervical protruded cervical mass. Colposcopy confirmed a tumor arising from the cérvix.

On T2-weighted imaging of pelvic magnetic resonance imaging (MRI), an enhancing tumor of the uterine cervix, sized 3.7x3.6x3.8 cm was found. The tumor was staged as IB1, according to the FIGO staging system 2014.

A conventional Pap specimen (Pap stain) was diagnosed as cellular changes consistent with carcinoma.

Disclosure of Interest: None declared.

SS01-6

Case 5

Takako Kiyokawa

Department of Pathology, The Jikei University School of Medicine, Japan

Case History: The patient is a 33 year-old Japanese woman who presented with abnormal uterine bleeding. A hysterectomy with bilateral salpingo-oophorectomy was performed after conization.

Disclosure of Interest: None declared.

SS02-1

Unusual Presentation on Pap Smear

Kusum Kapila

Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait

Method of Sample Collection: Conventional cervicovaginal smear obtained by scraping the squamo-columnar junction and an endocervical sample with a brush. Both the cervical and endocervical samples we were smeared on one slide and immediately flooded with a spray fixative.

Clinical Summary: A 50-year-old Kuwaiti female presented with postmenopausal bleeding and a pelvic abdominal mass. A complete physical examination revealed her to be hypertensive (blood pressure 180/110 mm Hg) and anaemic (Hb 5.7%; microcytic, hypochromic anaemia). On pelvic examination the uterus was enlarged corresponding to a 16-week gestational size. Ultrasonography revelaed a bulky uterus with ascites, bilateral hydronephrosis and polycystic ovaries. The liver and gall bladder appeared normal. The cervix was uniformly enlarged and firm with induration extending to the bladder and vaginal vault. On speculum examination the cervix had a pinpoint os, but no visible ulcer or tumour. A cervicovaginal smear was taken.

Disclosure of Interest: None declared.

SS02-2

Endometrial versus Cervical Cancer May Be Confused in Pap Tests: Will HPV Testing Help?

Amanda Herbert

Guy's and St. Thomas' NHS Foundation Trust, United Kingdom

Introduction: Endometrial carcinoma is rarely detected in asymptomatic women on cervical Pap smears or LBC preparations – and would be unlikely to be detected when primary HPV testing is in place. Furthermore, squamous cell carcinoma may be confused with endometrial carcinoma on Pap smears: accurate diagnosis depends on clinical correlation and follow up as well as cytological diagnosis. How much will HPV testing help?

Case History: Forty-nine-year-old woman first screened at thirty-three years of age. Cytology was reported as low-grade, negative (twice) and low-grade (twice) after which colposcopy resulted in a negative biopsy and cytology at age forty. The patient defaulted from invitations for follow-up cytology during the subsequent seven years. At age forty-nine, a routine Pap test was reported as atypical glandular cells. Pipelle was negative and the patient defaulted from cytology tests for a further two years.

The final diagnosis (concealed) was made two years later and will be considered in comparison with two cases with related problems.

Conclusion: Endometrial carcinoma may mimic squamous cell lesions and vice versa; cervical adenocarcinoma may be endometrioid in type; HPV testing may help in some instances but not others.

Disclosure of Interest: None declared.

SS02-3

A Case of High Grade Serous Carcinoma of Endometrium Diagnosed on an Endocyte Device and Confirmed by Histology

Franco Fulciniti, Jessica Barizzi, Elisabetta Merlo, Pierangela Grassi*

Istituto Cantonale di Patologia, Switzerland

A 70-year old woman with previous negative gynecological history underwent a routine gynecological visit as a part of a spontaneous triennial interval check up. Her external genitalia were normal for age and parity status, the cervix uteri was atrophic. A pap smear was taken, that was later processed as an LBC sample. Transvaginal ultrasound was performed with a multiparametric 6.3 Mhz probe which showed a normal sized uterus for age, with irregular thickening of the endometrial mucosa (8 mm). Endometrial sampling with and Endocyte sampler was performed and collected in cytolyt. The sample was later processed as an LBC sample. The cervical sample was negative for malignancy or intraepithelial lesions. Cytologic examination of the endometrial sample showed a high grade adenocarcinoma, which was thought to be of endometrial origin. The residual endometrial sample was centrifuged and a cell block was obtained by the plasma-thrombin method, from which 4–5 Uμ sections were obtained for Pap and H&E staining and immunocytochemistry. A cytological diagnosis of G3 serous adenocarcinoma was made which was later confirmed by Pipelle biopsy and radical hysterectomy.

Disclosure of Interest: None declared.

SS02-4

A Case of Endometrial Lesion in LBC Sample

Yoshinobu Maeda

Division of Diagnostic Pathology, Toyama Red Cross Hospital, Japan

A 80-year-old, 3G2P, postmenopausal Japanese woman was visited with a complaint of one week history of abnormal vaginal bleeding in October 2013. Although in ultrasound examination no remarkable findings were found, atypical endometrial gland cells composed irregular clusters were recognized in endometrial cytological examination. Examination material was smear obtained by Uterobrush from endometrial cavity, and was prepared by BD SurePath LBC method and Papanicolaou staining. A lab values for LDH, CEA, CA125 and CA72-4 were within normal range.

In December 2013, abdominal simple hysterectomy, bilateral salpingo-oohorectomy and pelvic lymphadenectomy were done. In uterine fundus, there was a dome-like tumor 1.5 cm in diameter. No metastasis of distant organs were not found and there were no pelvic lymph nodes metastases.

Disclosure of Interest: None declared.

SS02-5

A Challenging Case of Direct Endometrial Cytology

Chiung-Ru Lai[1,2]

[1]Department of Pathology, Taipei Veterans General Hospital, [2]National Yang Ming Medical University, Taiwan

Clinical History: A post-menopausal 70-year-old woman of G3P3A0 had no significant previous history. She had routine health check and transvaginal sonography showed thickened endometrium, up to 9 mm. D&C was suggested. However, scant tissue could be obtained. Direct endometrial cytology was performed instead, during the curettage procedure.

Cytology Specimen: Endometrial brush: 2 smears with Papanicolaou stain.

Disclosure of Interest: None declared.

SS03-1

Breast Case 1

Hirofumi Matsumoto

Department of Pathology, Ryukyu University Hospital, Japan

Age: 35
Gender: Female
Symptom(s) (or no symptoms): Left breast lump
Location: 9 to 11 o'clock
Size: 10 cm

Brief Clinical Info (including imaging findings): A 35-year-old Japanese lady presented with a rapidly growing left breast mass. On ultrasonographic examination, the mass had both solid and cystic areas. On the mammograms, the lesion was highly dense without calcifications.

Method of Sample Collection: Aspiration cytology.

Disclosure of Interest: None declared.

SS03-2

Breast Case 2

Naoki Kanomata[1]*, *Sakae Hata*[2], *Minako Fukuya*[1], *Takuya Moriya*[1]

[1]Department of Pathology, Kawasaki Medical School, [2]Department of Pathology, Kawasaki Medical School Kawasaki Hospital, Japan

Age: 44 year old
Gender: Female
Symptom: Breast lump and nipple discharge
Location: Right upper inner quadrant
Size: 2 cm

Brief Clinical Info: The patient had noticed a breast lump, and recently nipple discharge developed. An ultrasound study revealed a cystic lesion with solid nodule.

Method of Sample Collection: Fine needle aspiration cytology.

Disclosure of Interest: None declared.

SS03-3

Breast Case 3

Jen-fan Hang[1]*, *Yeh-han Wang*[2]

[1]Taipei Veterans General Hospital, [2]Keelung Hospital, Ministry of Health and Welfare, Taiwan

Age: 45 year-old
Gender: Male
Symptom: A right breast lump for 6 months
Location: Subareolar region of right breast
Size: 1.3 cm

Brief Clinical Info: The lump was painless, firm and non-movable. There was neither skin change nor palpable axillary lymph node. An ultrasonography showed an ovoid, isoechoic to hypoechoic nodule.

Method of Sample Collection: Under the suspicion of breast cancer, an ultrasound-guided aspiration was performed using a 23-gauge needle. Cytologic smears were made on glass slides, including 3 air-dried slides for Liu's stain and 3 alcohol-fixed slides for Papanicolaou stain.

Disclosure of Interest: None declared.

SS03-4

Breast Case 4

Ryuji Ohashi

Nippon Medical School Hospital, Department of Diagnostic Pathology, Japan

A 79-year-old woman presented with a mass in her left breast. Her past medical history was unremarkable. Ultrasound examination revealed an irregular mass measuring up to 2.2 cm in the lower outer quadrant of the left breast. Fine needle aspiration cytology biopsy was performed to determine the diagnosis of the lesion.

Disclosure of Interest: None declared.

SS03-5

Breast Case 5: A Case of Breast Tumor

Suzuko Moritani

Division of Diagnositic Pathlogy, Shiga University of Medical Science, Japan

Age: 50
Gender: Female
Symptom(s) (or no symptoms): Palpable mass
Location: Upper outer area of the right breast
Size: 25 mm

Brief Clinical Info (including imaging findings): A well circumscribed lobulated mass with coarse calcification in mammography.

Method of Sample Collection: Fine needle aspiration cytology.

Disclosure of Interest: None declared.

SS03-7

Breast Case 7

Fernando Schmitt

Laboratoire National De Santé, Luxembourg

Age: 63 year-old

Gender: Female

Symptoms: Palpable mass

Location: Upper quadrant, retroareolar region of the right breast

Size: 30×8 mm

Clinical Info: Bloody nipple discharge, poor circumscribed, cystic mass.

BIRADS 2 simple cysts in left breast and BIRADS 3 complicated cystic lesion in right breast.

Method of Sample Collection:

– nipple discharge

– right breast FNA

– right breast excisional biopsy

Disclosure of Interest: None declared.

SS04-1

Hashimoto's Thyroiditis Versus Papillary Thyroid Carcinoma

Yun Zhu, Tiesheng Wang*

Jiangsu Institute of Nuclear Medicine, China

This is a 35-year-old woman who was suffering from Hashimoto's thyroiditis for over 10 years. The patient was asymptomatic with high titers of thyroid peroxidase. Ultrasound images showed multiple hypoechoic areas varying in size in both lobes. A 7×3×4 mm solid hyperechoic nodule with micorcalcifications was detected in the left lobe. Fine needle aspiration (FNA) was performed and a diagnosis of Hashimoto's thyroiditis was rendered. After 21 month ultrasound surveillance, repeated FNA was performed due to growth of the nodule (2 mm in the maximum diameter) and the patient's strongly requirement as her mother underwent thyroid surgery owing to papillary thyroid carcinoma.

Disclosure of Interest: None declared.

SS04-2

Case Presentation. Thyroid FNA

Mitsuyoshi Hirokawa

Department of Diagnostic Cytology and Pathology, Kuma Hospital, Japan

Case: 48-year-old, woman

History: She was pointed out to have dyslipidemia by a medical checkup, and visited a hospital, where a nodule was demonstrated in the left lobe of the thyroid. She was referred to our hospital for close inspection.

Ultrasonographic Examination: Two nodules were found in the thyroid. The nodule located in the left lobe measured 26 mm x 17 mm x 24 mm. It was slightly hypoechoic and the internal echogenicity was homogeneous. The shape was slightly irregular, but the border was well-defined. The nodule was associated with multiple punctate hyperechogenic foci. Color Doppler ultrasound showed intra- and peri-tumoral hypervascular flow, called 'tumor inferno'. The nodule located in right lobe, measureng 10 mm x 6 mm x 6 mm, was interpreted as a benign nodule.

Our Presentation: Aspiration cytology for the left nodule.

Disclosure of Interest: None declared.

SS04-3

Uncommon Variant of Papillary Thyroid Carcinoma

Chiung-ru Lai

Department of Pathology, Taipei Veterans General Hospital, Taiwan

Clinical History: A 30-year-old man presented with a painless gradually enlarged thyroid. Ultrasound examination revealed multiple nodules in both lobes of the thyroid, with the largest one, 1 cm in the left lobe. Thyroid function was within normal limit. No other lesions in the head and neck area were identified.

Cytology Specimen: Fine needle aspiration of the largest nodule was performed and the aspirate was submitted to the cytology laboratory as one ethanol-fixed smear for Papanicolaou stain, one air-dried smear for Liu's stain, and one SurePath BD CytoRich™ vial for liquid-based preparation (Becton, Dickinson and Company) and cell block.

Disclosure of Interest: None declared.

SS04-4

Case Presentation

Zhiyan Liu[1,2]*, *Kennichi Kakudo*[3]

[1]Shandong University School of Medicine, [2]Shandong University Qilu Hospital, China, [3]Kindai University Faculty of Medicine, Japan

Brief Clinical Summary: The patient is a 70 years old female who had a long history of thyroid nodule in her left lobe. Elevated serum thyroglobulin was found and she was referred to our hospital for a further check-up.

Ultrasound: A well circumscribed thyroid nodule with rich vascularity is shown. It is a solid tumor with minor cystic change in the left lobe. No irregular margin suggestive for invasion into the thyroid parenchyma nor invasion to the thyroid capsule was found. No calcification was identified in the nodule.

Fine Needle Aspiration: Fine needle aspiration was done and papanicolaou stain was applied.

Cytopathology: Cytological images are available through HP.

Disclosure of Interest: None declared.

SS04-5

Diagnostic Pitfalls in PTC

Xin Jing

Department of Pathology, The University of Michigan Health System, United States

A 61 year-old female presented with neck mass. Ultrasound exam revealed multiple nodules in both thyroid lobes and isthmus. The prominant nodule was detected in inferior right lobe and measured 2.2 cm. Fine needle aspiration of the prominant nodule was performed with preparation of Diff Quick- and Pap-stained conventional smears.

Disclosure of Interest: None declared.

SS04-6

Molecular Diagnostics of AUS/FLUS Nodules: Present and Future

N. Paul Ohori

Department of Pathology, University of Pittsburgh Medical Center – Presbyterian, United States

The implementation of Thyroid Bethesda Classification System is standardizing the practice of Thyroid FNA cytology. Among the indeterminate diagnoses, the Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS) diagnosis is most challenging and controversial. The AUS/FLUS cases are limited in the abnormal features demonstrated and fall short of the Follicular Neoplasm/Suspicious for Follicular Neoplasm (FN/SFN) and Suspicious for Malignancy diagnoses; however, they show changes that exclude them from the Benign category. In general, these AUS/FLUS cases may be subcategorized further as those demonstrating a) architectural (microfollicular) atypia, b) cytologic (nuclear) atypia, or c) both architectural and cytologic atypia. Subsequent resection of these AUS/FLUS nodules often demonstrates follicular-patterned lesions [follicular adenoma, nodular hyperplasia, follicular variant papillary carcinoma (FVPTC), follicular carcinoma] or papillary-patterned lesions (papillary hyperplasia, papillary carcinoma). Given the relatively low risk of malignant outcome (5–15%) of these AUS/FLUS nodules, additional information from ancillary testing (e.g. molecular diagnostics) is needed to improve nodule characterization and risk stratification. Recently, the application of multi-gene molecular panel tests [e.g., ThyroSeq v2 Next Generation Sequencing (NGS), *Afirma*® Gene Expression Classifier (GEC)] has demonstrated remarkable results in this regard. For cases with the AUS/FLUS cytology diagnosis, a negative ThyroSeq v2 NGS or *Afirma*® GEC result would further decrease the risk of malignancy to 3% and 5%, respectively. Furthermore, a positive ThyroSeq v2 NGS result increases the risk of malignancy to 77%. While these results demonstrate the potential of molecular testing in triaging cases for medical or surgical management, they need be viewed in the context of upcoming changes in thyroid pathology. Among the follicular-patterned neoplasms, the non-invasive encapsulated FVPTC is being renamed as a non-malignant neoplastic entity. This shift will most likely decrease the risk of malignancy for the negative and positive molecular results. On the other hand, mutations such as *RAS, PAX8-PPARγ, BRAF* K601E, *EIF1AX, GNAS, PTEN, TSHR, STRN-ALK,* and *EML-ALK* have become associated with follicular-patterned neoplasms and the correlation of mutations with subtypes of AUS/FLUS can provide insight into lesional characteristics. The role of thyroid cytology and molecular testing will continue to evolve with upcoming developments.

Disclosure of Interest: None declared.

SS04-7

Differential Diagnosis and Diagnostic Pitfalls of Cystic Lesions of the Thyroid

Claire W. Michael

Case Western Reserve University/University Hospitals Case Medical Center, United States

Fine needle aspiration of a cystic thyroid nodule is very common. These aspirates frequently consist of numerous macrophages that may or may not contain hemosiderin. Because of the risk of papillary carcinoma, the presence of macrophages only does not constitute an adequate specimen. Despite the similar morphology, the differential diagnosis can range from a benign simple cyst to cystic papillary carcinoma with very few representative malignant cells on the slide.

This session will discuss the range of morphology encountered in cystic lesions and outline the diagnostic pitfalls and clues to the diagnosis.

In addition, the presenter will review the diagnostic pitfalls and features of thyroid aspirates prepared by Liquid Based Preparations (ThinPrep and SurePath).

Disclosure of Interest: None declared.

SS05-1

Method of Sample Collection for Cytology; Voided Urine

Jee-young Han

Department of Pathology, Inha University Medical College, Korea

Clinical History: A 72-old-male was admitted to our hospital because of intermittent gross hematuria for 1 year. He was diagnosed with benign prostate hyperplasia 14 months ago. The CT urography showed generalized wall thickness and irregularly enhancing lesion in the urinary bladder. Serum PSA level was normal (0.124 ng/mL). Routine urinalysis test reveled 1+ protein, 4+ red blood cells, and 1+ white blood cells. The results of blood chemistry were within normal limits. For diagnosis, voided urine cytology was performed.

Disclosure of Interest: None declared.

SS05-2

Urine Cytology, Case 2

Hiroshi Ohtani[1], Yuko Koide[1], Ken-ichi Mori[1], Nobuyoshi Terado[2], Yoshinao Oda[2], Masatoshi Eto[2], Mieko Hakuraku[3], Masami Kudo[3], Kanae Sekimoto[3]*

[1]Hakujyuji Hospital, [2]Kyushu University, [3]Research Institute of Clinical Pathobiology, Japan

A 66-year-old Japanese male presented at a hospital with complaints of gross hematuria over six months. Prior to this he had been asymptomatic. Voided urine samples were processed using CytoRich Red (Becton Dickinson).

Disclosure of Interest: None declared.

SS05-3

Urothelial Cells or Not?

Shin-ichi Murata

Departments of Human Pathology/Diagnostic Pathology, Wakayama Medical University, Japan

Clinical History: A 69 year-old man presented gross hematuria. He had medical history of nephrotic syndrome for 6 years. Void urine cytology was done with cytospin preparations and Papanicolaou stain.

Choices for Diagnosis:

1. Reactive urothelial cells
2. Renal tubular epithelial cells
3. Urothelial carcinoma
4. Prostatic adenocarcinoma
5. Seminoma

Disclosure of Interest: None declared.

SS05-4

Urine Cytology – Preserving the Evidence

Jason Stone

QML Pathology, Australia

Key points:

- SurePath technology can be used for urine cytology.
- The degree of preservation and degeneration of the sample prior to receipt by the laboratory can significantly alter the morphology of the diagnostic cells
- Prostate cancer can be seen in urine cytology
- High Gleason score prostate carcinoma can mimic urothelial carcinoma
- Clues of glandular differentiation should be looked for
- Molecular techniques for diagnosing prostate cancer can be applied to urine samples.
- Testing for mRNA of the PCA3 gene is an FDA approved test for prostate cancer on urine samples
- Emerging techniques of assessing microRNA particles in urine, that are associated with prostate cancer are being developed and may provide a useful non-invasive tool for assessing the epigenetic phenomena in prostate cancer.

Disclosure of Interest: None declared.

SS06-1

A 46 Year-Old Female, Pleural Effusion

Yuko Minami[1], Yukinobu Goto[2], Yoshigiko Murata[3], Tomoki Nakagawa[3], Hiromi Fujiwara[3], Masayuki Noguchi[4]*

[1]National Hospital Organization Ibarakihigashi Hospital, The Center of Chest Diseases and Severe Motor and Intellectual Disabilities, Pathology Department, [2]University of Tsukuba, Faculty of Medicine, Thoracic Surgery, [3]University of Tsukuba Hospital, Pathology Department, [4]University of Tsukuba, Faculty of Medicine, Diagnostic Surgical Pathology, Japan

Case: Chief complaint: Right femoral pain/

Sample Collection for Cytology: Pleural effusion, Liquid based biopsy/

Clinical Summary: A 46 year-old female visited a local hospital with right femoral pain.

CT scan revealed pancreatic tumor, multiple mediastinal lymph nodes metastasis, multiple liver metastases, multiple bone

metastases, multiple brain metastases, multiple lung metastases, right pleural dissemination and right pleural effusion. Right pleural effusion was punctured.

Disclosure of Interest: None declared.

SS06-2

Interrater Agreement of Sputum Cytology for Lung Cancer Screening in Japan

Chiaki Endo[1]*, *Ryutaro Nakashima*[2], *Akemi Taguchi*[3], *Kazunobu Yahata*[4], *Ei Kawahara*[5], *Nikako Shimagaki*[6], *Junko Kamio*[7], *Yasuki Saito*[8], *Norihiko Ikeda*[9], *Masami Sato*[10]

[1]Sendai Tokushukai Hospital, [2]Miyagi Cancer Society, [3]Chiba Foundation for Health Promotion and Disease Prevention, [4]Osaka Medical Association, [5]Kanazawa University, [6]Niigata Health Service Center, [7]Fukushima Preventive Service Association of Health, [8]Sendai Medical Center, [9]Tokyo Medical University, [10]Kagoshima University, Japan

Objectives: Sputum cytology affords a simple, noninvasive assessment of central type lung cancer including premalignant lesions. In order to compare lung cancer detection rate by sputum cytology, we need some assurance that the estimates do not vary widely even if the different observers evaluate the same sputum cytology slides. However, to date, we have never studied the interobserver agreement of sputum cytology well in Japan. In order to elucidate the variability of sputum cytology diagnosis, we proposed the idea of the standardized sputum cytology cases and conducted a study to evaluate the agreement of sputum cytology diagnosis.

Methods and Results: Firstly, we presented the idea of the standardized sputum cytology slides, which any experienced observers diagnosed as the same category. Secondly, by using the standardized sputum cytology slides, we showed the agreement among observers of less experience of sputum cytology showed significantly lower than that among those of much experience, and observers of less experience tended to underestimate their evaluation.

Conclusion: We concluded only experienced observers should evaluate sputum cytology slides in order to diagnose accurately.

Disclosure of Interest: None declared.

SS06-3

A Case of Lung Cancer

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A 60-year-old Japanese man who had a history of smoking (20 cigarettes a day for 30 years) was admitted to our hospital complaining of a persistent cough and bloody sputum. A chest computed tomography confirmed a mass measuring about 30 mm in the left hilum of the lung with bilateral hilar and mediastinal lymphadenopathy. Positron emission tomography (PET) revealed 2-deoxy-2-^{18}F-fluoro-D-glucose (FDG) uptake in lung lesion, and multiple foci of the brain, liver, and bone. Thus, the patient was diagnosed clinically with carcinoma of the lung, cT4N3M1b, stage IV. To clarify the nature of the lesion, a bronchial endoscopic examination was done and revealed bronchial stenosis by tumor mass associated with bleeding tendency. Transbronchial brush cytology and transbronchial lung biopsy (TBLB) were also performed. Direct smears are prepared and stained with Papanicolaou stain from material obtained by brushing cytology.

Disclosure of Interest: None declared.

SS06-4

Cytologic Characteristics of Pulmonary Adenocarcinomas with Genetic Abnormalities

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The Method of Sample Collection for Cytology: Papanicolaou-stained transbronchial aspiration cytology (TBAC).

Clinical Summary:

Case 1. A Japanese male in the seventies, who was a non-smoker, presented with a lung nodule found at a postoperative follow-up examination for prostate cancer. Computed tomographic examination revealed a 21-mm-sized nodule in the upper right lung field. TBAC and biopsy from the nodule were performed. The nodule was resected with a right upper lobectomy.

Case 2. A Japanese male in the fifties, who was a non-smoker, presented with a lung nodule, found on a chest x-ray film at a regular health checkup. Computed tomographic examination revealed a 41-mm-sized nodule in the upper left lung field. TBAC and biopsy from the nodule were performed. The nodule was resected with a left upper lobectomy.

Disclosure of Interest: None declared.

SS07-1

Low Grade Lymphoma

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Method: Liquid based cytology, fine needle aspiration.

Clinical Findings: A 82-year-old man.

He noticed a left parotid gland swelling, and consulted a doctor. Since the lesion was clinically suspected to be parotid gland tumor, he was performed fine needle aspiration biopsy from left parotid gland.

Disclosure of Interest: None declared.

SS07-2

Low Grade Lymphoma and Related Lymphoproliferative Disorder

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The patient was an 83-year-old Japanese male who underwent total prostatectomy in another hospital eight years previously due to prostatic cancer. During the follow-up period, bilateral cervical lymph node swelling, 2 to 3 cm in diameter, was observed, thus he was admitted to our hospital. Laboratory examinations showed WBC 5000/μl, RBC 3.28×10^6/μl, PTL 26.1×10^4/μl, LDH 176 U/l and IL-2R 2580 U/ml. Fine needle aspiration (FNA) cytology from the cervical lymph node was performed because malignant lymphoma was suspected.

Disclosure of Interest: None declared.

SS07-3

Slide Seminar 7: Low Grade Lymphoma and Related Lymphoproliferative Disorder

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The patient was a 70s Japanese male. He visited a local hospital with a complaint of abdominal discomfort and was pointed out abdominal mass. Then, he admitted to our hospital for examinations. Superficial lymph nodes were not palpable. Abdominal CT revealed an 8 cm-sized tumor which is ventral to pancreas and multiple lymphadenopathy. Laboratory examination showed WBC 6.5×10^3/μL, RBC 4.69×10^6/μL, PLT 24.4×10^4/μL, LDH 207 IU/L and IL-2R 1,320 U/mL. Hypertension and hyperuricemia in past history and no appreciable disease in family history were present. For cytological and histological diagnosis, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was performed.

Disclosure of Interest: None declared.

SS07-4

Low Grade Lymphoma Presenting as Ascites and Pleural Effusion Accompanied by Leukemic Dissemination

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28-year-old-male visited the gastroenterology outpatient clinic complaining abdominal distension and dyspnea for 2 months. The blood cell count and laboratory findings were as follows; WBC 605.57×10^3/ml, Hb 9.7 g/dL and platelet 140×10^3/ml, AST/ALT 108/22 IU/L, LDH 265 IU/L, and alkaline phosphatase 276 IU/L. Abdominal computed tomography (CT) scan revealed multiple lymph node (LN) enlargement in the abdomen, pelvis, bilateral iliac and inguinal areas, splenomegaly, ascites, and large amount of bilateral pleural effusion. Whole body PET revealed conglomerated multiple hypermetabolic LNs along the lymphatic chain in both neck, both axilla, mediastinum, omentum, retroperitoneum, both internal/external iliac and inguinal area. Bone marrow examination revealed hypercellular marrow with diffuse infiltration of abnormal lymphoid cells (97.4% of nucleated cells) (cellularity: 91–100%). The patient was admitted for the clinical impression of lymphoma or leukemia. Cytology was submitted from thoracentesis of right pleural effusion, and excisional biopsy was done at left inguinal lymph node. The submitted slide is Papanicolaou-stained cytology smear slide from pleural fluid.

Disclosure of Interest: None declared.

SS07-5

Case Presentation on Low Grade Lymphoma and Related Lymphoproliferative Disorders

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Clinical Details: A 15 year old male patient presented with a right cervical lymph node swelling slowly increasing in size for six months. There were no other symptoms or any systemic complaints like fever or malaise. On examination, there was a 3 cm diameter cervical lymph node.

Materials and Methods: A fine needle aspiration was performed from the lymph node as an outpatient procedure using a 23 gauge needle and a 10 ml syringe fitted to a syringe holder. One alcohol fixed smear was stained for Papanicolaou stain and one air dried smear for May Grunwald Giemsa were prepared.

Results: Microscopic examination showed lymphoid tissue with germinal center cells. Vascular fragments with perivascular clustering of lymphoid cells was prominent. Many large cells with large nuclei and prominent nucleoli were identified including a few binucleated forms. An inconclusive report with suspicion of

Hodgkin's lymphoma was given. A lymph node biopsy was advised.

Conclusion: Case for discussion.

Disclosure of Interest: None declared.

CM01-1

Glandular Lesions in Pap Smears-Endocervix, Endometrium, and Extra-Uterine

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Background: The Papanicolaou smear has been successfully used for the screening of cervical squamous premalignant and malignant lesions and subsequently reduced the incidence and mortality of cervical cancer. Unfortunately, the incidence of endocervical, endometrial and ovarian adenocarcinoma has paradoxically risen in these years. The purpose of the talk was to introduce some morphological observations highly suggestive of significant pathology to increase the sensitivity of the interpretation of Pap smears for glandular lesions.

Hints of Endometrial Origin: Degenerative atypical glandular cells admixed with watery diathesis, foamy histiocytes, cell debris, and marked cellular degeneration with phagocytosis.

Hints of Endocervical Mucinous Adenocarcinoma: Necrotic and mucinous background resembling those of ileal conduit urine was a suggestive indicator for endocervical mucinous adenocarcinoma.

Hints of Extra-Uterine Origin: Clean background, abnormal cells in 3-D cell balls not relevant to the background, frank high-grade adenocarcinoma cells.

Take Home Message:

a) Although it is generally accepted that the aim of Pap test is primarily to detect squamous lesions, especially the precursors.

b) However, if we do notice the additional fairly characteristic cytomorphology pictures existed in the Pap smears warranted further and precise evaluation which we've already got, it really could help the clinicians and patients a lot.

c) Why not start to devote more efforts to recognize the endocervical, endometrial and extra-uterine adenocarcinomas.

Disclosure of Interest: None declared.

CM01-2

Pitfalls in Lung Cancer Cytopathology Diagnosis: Cipto Mangunkusumo Hospital/FMUI, Jakarta, Indonesia Experience

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Introduction: Diagnostic pathology of lung cancer is important in the era of the development molecular target therapy in lung cancer in determining the optimal therapy. Cytopathology also plays an important role in diagnosis of lung cancer, particularly since molecular profiles can be analyzed using cytology specimens. The aim of this study was to discuss lung cancer cytopathology cases and its pitfalls.

Material and Methods: Lung cytopathology cases from lavage, brushing, TTNA and EBUS TBNA which have the histopathology and or immunocytochemistry/immunohistochemistry follow up were sought from the FMUI/CMH, Anatomical Pathology department archive. Cytomorphology findings were analyzed according the WHO classification diagnosis of lung cancer. The clinical data congruity and the cytomorphology findings were reviewed in discordance and or difficult cases.

Results: There were classic and non-classic cytomorphology features of Squamous cell carcinoma, adenocarcinoma and neuroendocrine tumor, etc. The non-classic features were difficult to determine only by cytomorphology and immunocytochemistry was needed. There were also rare or interesting cases such as adenoid cystic carcinoma and mesenchymal chondrosarcoma.

Conclusion: Clinicopathological conference and ancillary testing are important in establishing the diagnosis of cytopathology lung cancer.

Disclosure of Interest: None declared.

CM01-3

The Implication of Atypia of Undetermined Significance (AUS) Category to a Pathologist in a Third World Country

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AUS has been the most discussed category in the current Bethesda thyroid fine needle aspiration (FNA) classification because of the heterogeneity of its use. Most studies have focused on the possibility of stratifying this category since different patterns of AUS/FLUS may carry different risks of malignancy, while other authors have put forth the idea that this category can be eliminated.

In certain areas of the Philippines it was observed that over a five year period, there were a total of 3,799 thyroid FNA cases, of which 207 (5.45%) were diagnosed as AUS. Of these 207 patients 33 (16%) who underwent surgery, 20 (61%) turned out benign and 13 of 33 (39%) cases were malignant on histology. 174 (84%) thyroid AUS cases were lost to follow-up.

With a higher risk of malignancy of AUS category in this study compared to the TBSRTC data, of utmost concern is the greater number of AUS cases who were lost to follow up (174 patients in this sample). In the Philippines, as it is in other low- and middle income countries, not everyone have access to good health care. Most people have poor health-seeking behavior and find frequent visits and close follow up difficult to adhere to because of attitude and of the extra burden of additional expenses. Thus in order to save lives, it is recommended that the number of AUS cases be decreased by strict adherence to criteria in diagnosing thyroid aspirates on the patients' first visit.

Disclosure of Interest: None declared.

CM01-4

Early Detection Program of Uterine Cervical Cancer in Mongolia

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Background: Mongolian Health Ministry approved the 'Clinical Practice Guideline for Uterine Cervical Cancer' in 2011, which is requested to have Papanicolaou (Pap) smear screening for all Mongolian women aged 30–60 years. Guidelines on frequency vary from every three years, if the result of Pap smear is negative. However, colposcopy guided tissue biopsy is recommended to confirm the final pathological diagnosis when the result of a Pap smear is abnormal. Therefore, to demonstrate that an impact of cervical cancer screening program based on the Pap smear results in Mongolian.

Results: In last 20 years, the incidence rate shows that there are around 26.9 new cervical cancer cases for every 100,000 females in the Mongolia from 1991 to 2010 ($p < 0.0001$). The incidence of cervical cancer shows that 60.6% occur in women aged 40–59 years ($p = 0.001$). In 2010, stage of cervical cancer were follows; 0 = 15.6%, I = 13.4%, II = 13.4%, III = 50.4%, and IV = 6.9%.

In last 3 years, the completion of early detection program is 42% in Mongolia. During this time, 220598 Pap smear was screened, among them 286 (0.13%) smear is reported cervical neoplasm. The stage of cervical cancer were follows; 0 = 43%, I = 20.6%, II = 12.7%, III = 17.7%, and IV = 5.3%. The incidence rate shows that there are around 28.8 new cervical cancer cases for every 100,000 females in the Mongolia (2012–2014). In Ulaanbaatar city, the cytological diagnosis concluded as follows; 53.2% were ASCUS, 10.0% were ASCH, 19.2% were LSIL, 13.4% were HSIL, 3.4% were CIS, and 0.8% was SCC.

Conclusions: After the implementation of early detection program in cervical cancer, most of cervical cancer diagnosed in early stage compare to the old time. In addition, the incidence of cervical cancer is increased due to population based screening program.

Disclosure of Interest: None declared.

CM01-5

Fine Needle Aspiration Cytology of Hepatocellular Carcinoma: Future Perspective

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Hepatocellular carcinoma (HCC) is a burgeoning global problem with high mortality. The majority are associated with cirrhosis due to different etiologies. Hepatocarcinogenesis is complex. The heterogeneity and molecular diversity of HCC make it a diagnostic and therapeutic challenge. A molecular subclassification with clinicopathologic correlates for the purpose of enhancing management practices is imminent. Molecular profiling of HCC has implications on diagnosis of early lesions, prognostication, and identification of therapeutic agents for personalized molecular targeted therapy.

The current practice of noninvasive diagnosis of HCC based on classic imaging features may no longer suffice. It is becoming increasingly important to obtain biopsy tissue for morphologic, immunohistochemical and molecular studies. Fine needle aspiration biopsy (FNAB) and/or needle core biopsy (NCB) is the best foreseeable option to obtain fresh tumor and peritumoral tissue; despite the shortcomings/limitations of the technique/small tissue samples. Increasingly, molecular studies can be performed on formalin-fixed paraffin-embedded tissue.

Biopsy is recommended in radiologically atypical liver nodules, small nodules with indeterminate radiologic features, and those occurring in non-cirrhotic livers to exclude benign lesions and intrahepatic cholangiocarcinoma. In small hepatocellular nodules (2 cm or less), HCC has to be distinguished from high grade dysplastic nodule for priority in liver transplant listing. Extended HCC recipient criteria for transplant require exclusion of high grade components. HCCs planned for non-surgical treatment options such as locoregional ablation therapies and sorafenib should optimally be biopsied.

Cancer classification aims to establish prognosis and select appropriate treatment. Tumor grade or differentiation, histologic variations, CK19 status, and molecular biomarkers are prognostic variables attainable from FNAB. Being minimally invasive FNAB allows for multiple passes, using co-axial approach, to achieve better sampling of large/heterogeneous tumors, and to sample peritumoral tissue. Molecular study of non-tumoral tissue may hint at likelihood of tumor recurrence.

Aspiration of tumor cells for identification of molecular predictors of drug response (responders) is cost-effective. Tumor cells for drug testing for response to single/combination therapeutic agents in 'liver (tumor) on a chip' model may be in the pipeline. The advent of genomic profiling strongly heralds the reinstate-

ment of FNAB as a point of care in the diagnosis, prognosis prediction, and treatment of HCC.

Disclosure of Interest: None declared.

CM02-1

National Cervical Cancer Screening Program by Conventional Cytology: 10 Years of Experience from Thailand

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Cervical cancer is the second most common cancer in Thai women. In response to this, a National cervical cancer screening program wasestablishedwith phase I running from 2005–2009 and phase IIfrom 2010–2014. The first phase of the program covered women aged from 35 to 60 and we expected to screen 10 million women. The actual number screened was 2,036,018 with 1,491 cases of carcinoma, 9,169 cases of HSIL and 11,551 cases of LSIL identified. In the second phase we included women aged from 30 to 60 and we expected to screen 13 million women. The actual number screened was 7,637,226 with 3,669 cases of carcinoma, 30,761 cases of HSIL and 67,125 cases of LSIL identified. The coverage of both phases was only 20% and 59% respectively. To improve the program we investigated new screening procedures that would improve coverage (number of women screened) and the quality of the cytology slides. To achieve this we carried out two pilot studies. In the firststudy, cervical smears were collectedin aliquid-based preservative and triagedusing HPV testing: HR-HPVpositive samples were then examined usingliquid-based cytology. In the second study, smears were triaged using urine HPV testing: positive sampleswere followed upwith cervical HPV testing and liquid-based cytology. Results were impressive; both techniques increased the coverage and sensitivity of testing and we are now presenting both procedures to the National Health Security Office for selection and implementation.

Disclosure of Interest: None declared.

CM02-2

Cervical Cancer Screening Program in Hong Kong and China: Present and Future

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Globally, cervical cancer is the fourth most common cancer among women in 2012. In Hong Kong, cervical cancer is the seventh most common cancer and the ninth most fatal cancer in 2013. Hong Kong can be regarded as a successful example of opportunistic screening for cervical cancer. Cervical cytology test became available from the 1960s followed by decline in incidence. There is established screening guideline and accreditation of smear takers, colposcopists and cervical cytology reporting laboratories is encouraged. Liquid based cytology is widely used and imager assisted screening is available. Molecular test for high risk human papillomavirus (HPV) is increasingly adopted for triage of atypical squamous cells of undetermined significance and primary screening. However, screening uptake patterns in Hong Kong is still suboptimal with about 70% of our women aged 25–64 have ever had cervical smear. Women who participate in regular screening tend to be younger and more socioeconomic advantaged. While both bivalent and tetravalent vaccines are available in Hong Kong, they are not included in the government funded vaccination program although the government is considering a pilot HPV vaccination program for girls from low income families.

In mainland China, cervical cancer is also one of the most common malignancies in women. The incidence and mortality of cervical cancer vary widely by geographic area, population and time period. While a declining trend in mortality was observed in most areas, it was less significant in rural compared to urban areas. It is also alarming to notice a more severe risk of cervical cancer in younger women. A recent study using data from the 2010 China Chronic Disease and Risk Factor Surveillance System showed that 21% of women had ever had a cytology test, less often among women in rural areas. Factors associated with taking cytology test were being aged 30–49 years, higher education, being married, and having urban health insurance. Since the beginning of this century, the Ministry of Health and All-China Women's Federation have been active in launching cervical cancer screening particularly in rural China, utilizing free visual inspection with acetic acid/iodine solution or cytology tests. However, it is estimated that the total number of rural Chinese women in the target age group (35–59 years) who need cervical cancer screening is around 142 million. As in other countries, such screening is also limited by the capacity of current health service. It is proposed that HPV DNA testing and self-sampling should be adopted to improve sensitivity and participation.

Disclosure of Interest: None declared.

CM02-3

Screening Strategy in Vaccinated Population: Australian Experience

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The high rate of coverage that has been achieved to date by the Australian government's Human Papillomavirus (HPV) Vaccination Program has already led to profound reductions in the prevalence of biopsy-confirmed, high-grade abnormalities and of vaccine-preventable HPV types in Australia. Declines in the prevalence of vaccine preventable HPV have occurred not only in vaccinated women but also in unvaccinated women, suggesting a herd-immunity affect. These declines were anticipated on the basis of modelling and were the major drivers for the changes proposed to the Australian National Cervical Screening Program. The federal and state-based Australian governments established a "Re-

newal Steering Committee," which conducted a literature search and a review of the available evidence to assess its applicability and quality. Together with this information the committee also used modeling to determine the optimal screening pathway for cervical cancer screening and constructed a plan for implementing the changes that will be required to transition from the currently successful screening program to the renewed program. The committee recommended that Australia move to a screening program based on testing every 5 years using an HPV test with partial genotyping with reflex liquid-based cytology (LBC) triage for HPV-vaccinated and unvaccinated women ages 25 to 69 years, and an additional exit test for women up to age 74 years. Primary HPV testing and reflex LBC will be funded by government. Symptomatic women outside the screening program will also be able to access government funded testing.

CM02-4

Cytological Features of Atypical Squamous Cells of Undetermined Significance and Glandular Abnormalities in the Conventional Papanicolaou Smears and Liquid-Based ThinPrep Specimens

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Objective: To clarify the cytological features of atypical squamous cells of undetermined significance (ASC-US) and glandular abnormalities of the uterine cervix in liquid-based ThinPrep method.

Methods: A total of 11,039 cervical samples enrolled from multiple hospitals in Japan were analyzed using a split-sample method via conventional Papanicolaou (CP) and liquid-based ThinPrep (TP) methods. Cytological features of ASC-US and glandular abnormalities were highlighted to compare histological diagnoses, and all positive samples were examined for their HPV prevalence using a Multiplex PCR method.

Results: Positive samples (ASC-US or more advanced) were found in 10.7% by CP and 10.3% by TP with no statistical difference. ASC-US was detected more frequently ($p < 0.0001$) by CP and high grade squamous intraepithelial lesion or more advanced were detected more frequently ($p < 0.001$) by TP compared to another method. Cytological features of ASC-US by CP showed characteristically the qualitatively insufficient nuclear atypism due to dehydration or degeneration. Conversely, those by TP showed an increased nucleus/cell ratio with slightly increased chromatin. On the other hand, glandular abnormalities including atypical glandular cells, adenocarcinoma in-situ, adenocarcinoma and adenosquamous cell carcinoma were detected in the identical frequency (67 samples by CP, 69 samples by TP, 0.6% in frequency each).

Conclusions: To diagnose ASC-US and glandular abnormalities correctly by TP method, their definition and characteristic cytological features must be understood and learnt clearly. The different features caused by different methods should not be underestimated.

Disclosure of Interest: None declared.

CM03-1

History of the K-J Joint Meeting of Diagnostic Cytology

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Since the First Korea-Japan Joint Meeting for Diagnostic Cytology was started at the Anam Hospital of Korea University Medical Center in 2002, the 14th Meeting was successfully held in Yeosu MVL Hotel, Jeollanam Do of Republic of Korea at the 7th, November 2015. Except the 12th Meeting in 2013, which was held in conjunction with the 9th Asia-Pacific IAP Meeting in Busan, all this Joint Meeting has been scheduled on the next day of the Fall Congress of the Korean Society for Cytopathology, and consists of oral presentations and poster presentations from both countries. During 13 Meetings, 52 oral presentations and 327 poster presentations were discussed, and 332 Japanese participants attended the Meeting. This Korea-Japan Joint Meeting for Diagnostic Cytology brought us much knowledge of cytopathology and friendship between two countries, As one of the members who initiated this Joint Meeting and as the First Meeting Organizer, I will review and propose the future direction of this Joint Meeting.

Disclosure of Interest: None declared.

CM03-2

Aberrant DNA Methylation of DLX4 and SIM1 Genes Is a Predictive Marker for Disease Progression of Uterine Cervical Low-Grade Squamous Intraepithelial Lesion

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Background: Cervical cancer is the second-most common cancer in women worldwide. In Japan, it was the 11th leading cause of death from cancer for women in 2008 but there has been a unique increase of cervical cancer incidence among women in the 20–39-year age group in spite of the decrease of it among women over age 40. Aberrant DNA methylation (abMet) is shown to provide disease biomarkers with great potential applicable to clinical specimens. The authors examined the relationship between the abMet of *DLX4* and *SIM1* genes and progression of low-grade squamous intraepithelial lesion (LSIL).

Methods: A total of 113 patients were selected from the CCLBC study, in which 11,039 samples were enrolled between October 2007 and March 2010 to compare the cytological features of conventional Pap smears and liquid-based cytology specimens using the ThinPrep method. They were classified into four groups according to their cervical cytology, HPV infection and follow up. Cytology samples were examined for abMet of *DLX4* and *SIM1* genes and their protein expressions. CaSki cells were treated with 5-Aza-2'-deoxycytidine (5-aza-dC).

Results: 40 samples in Group 1 were negative for intraepithelial lesion or malignancy. 21 LSILs in Group 2 showed a continuance of LSIL for longer than 365 days, and 12 LSILs in Group 3 showed an up-grading to high-grade (H) SIL+ within 365 days after the diagnosis of LSIL. 40 in Group 4 were squamous cell carcinoma. All but Group 1 were infected with hrHPV. Significant difference existed in frequency of abMet between groups 2 and 3 ($p = 0.044$), between groups 3 and 4 ($p = 0.020$) for *DLX4*, and between groups 1 and 3 ($p = 0.0003$), as well as between groups 2 and 3 ($p = 0.005$) for *SIM1* gene. *DLX4* protein expression was significantly reduced in the *DLX4* abMet positive tissues, as compared to the negative tissues ($p = 0.008$), and 5-aza-dC treatment extracted *DLX4* protein expression of CaSki cells in a dose-dependent manner ($p < 0.005$). The LSIL cases with abMet of *SIM1* gene or both genes progressed faster to HSIL+ than others ($p = 0.033$ or $p = 0.048$).

Conclusion: AbMet of *DLX4* and *SIM1* genes should be a useful and novel progression marker of uterine cervical LSIL with HPV infection.

Disclosure of Interest: None declared.

CM03-3

Cervical Cancer Screening in Korea: Cytopathology in Update of the National Guideline Recommendation

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The incidence rate of cervical cancer in Korea is still higher than in other developed countries, notwithstanding the national mass screening program. For more than 10 years, cytopathologists and cytotechnicians in Korea have been responsible for the conventional cervicovaginal cytology (CCC) specimens in this program, where liquid-based cytology (LBC) were not formally allowed. The accuracy of the CCC were considered as higher than expected, however, a new method has been introduced in cervical cancer screening. Therefore, the committee for cervical cancer screening in Korea updated the recommendation statement established in 2002.

The committee were composed of gynecologists, cytopathologists and doctors of preventive medicine and family medicine. The new version of the guideline was developed by the committee using evidence-based methods. The committee reviewed the evidence for the benefits and harms of the CCC, LBC and human papilloma virus (HPV) testing, and reached conclusions after deliberation.

The committee recommends screening for cervical cancer with cytology (CCC or LBC) every three years in women older than 20 years of age (recommendation A). The combination test (cytology with HPV test) is optionally recommended after taking into consideration individual risk or preference (recommendation C). The current evidence for primary HPV screening is insufficient to assess the benefits and harms of cervical cancer screening (recommendation I). Cervical cancer screening can be terminated at the age of 74 years if more than three consecutive negative cytology reports have been confirmed within ten years (recommendation D).

This was the first experience for the Korean Society for Cytopathology to participate in updates of the national screening program for uterine cervical cancer, providing evidences for efficacy of CCC.

Disclosure of Interest: None declared.

CM03-4

Consciousness and Knowledge of Cervical Cancer Screening and HPV Vaccine in Japanese Young Adults

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In Japan, cervical cancer develops in approximately 10,000 females annually, leading to nearly 3,000 deaths each year. The incidence is increasing and particularly marked among those in their 20s and 30s, drawing social attention to young and parenting females' loss of fertility life due to cervical cancer as a serious issue. Despite this situation, awareness about cancer screening is still low in Japan. While 70–80% of people take screenings in Europe and the United States, only 20–30% do so in Japan. In 2009, Japanese government improved cervical cancer screening by announcing a new five-year program to send free-coupon to women aged 20, 25, 30, 35 and 40. In Japan, Human Papilloma virus (HPV) vaccine was approved in 2009, but indeed Japan's health ministry issued a nationwide notice that cervical cancer vaccinations should no longer be recommended due to several hundred adverse reaction to the vaccines reported.

Young people often find it difficult to obtain clear and correct information on issues that concern them such as risk of sexually transmitted infections (STIs) including HPV and relation between HPV and cervical cancer. Therefore peer education is one of the effective ways of dealing with these issues.

We introduce the consciousness and knowledge of cervical cancer screening and self-sampling of Japanese young adults. A sample of 144 university students, 115 women and 29 men, aged 19–23 completed self-administered pencil-and-paper questionnaires with approximately 30 questions as follows. A pre and post interventional survey were carried out regarding knowledge of HPV, cervical cancer and vaccination, experience of cancer screening program, vaccination status, on the students before and after peer educational interventions.

From the overall analysis of the data, it is revealed that the intervention produced some positive effects. After intervention the consciousness of own health protection became more closely linked with their behaviors, lifestyles and their own choices more than before, Interestingly for men, peers' was also important and effective. Our data highlights that a better understanding of HPV infection and possibility of preventing cervical cancer through cancer screening program and HPV vaccination, can increase the acceptance of the young by peer education.

Disclosure of Interest: None declared.

CM03-5

Papillary Thyroid Carcinoma and Its Variants in Fine Needle Aspiration Cytology

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More than ten histopathologic variants of papillary thyroid carcinoma (PTC) have been described. Some of the variants have a better prognosis than the classic PTC, while others behave in an aggressive fashion, with a higher frequency of morbidity and mortality. The variants with more unfavorable outcomes are the tall cell, columnar cell, and hobnail variants. Patients with these aggressive variants are classified into the intermediate-risk group regardless of tumor size or status of lymph node metastasis. Therefore, preoperative confirmation of aggressive variants on fine needle aspiration would be helpful to determine the type and extent of surgery. The prognostic implication of the solid variant and diffuse sclerosing variant may be associated with a less favorable outcome, but remains controversial. The encapsulated follicular variant of PTC is, in contrast, associated with a low risk of recurrence, particularly in the absence of capsular or vascular invasion. The cribriform-morular variant of PTC is frequently seen in patients with familial adenomatous polyposis. In this talk, I will present our studies of cytologic diagnosis of clinically important variants of PTC.

Disclosure of Interest: None declared.

CM04-1

Cervical Screening in Australia: A Great Success Story

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Australia has had a successful cervical screening program for over 20 years. Data from the Australian Institute of Health & Welfare show that the incidence of and mortality from cervical cancer have fallen by more than half during this time. How has this been achieved?

In the late 1980's, it was recognised both in Australian and worldwide, that for screening to work optimally, the screening program had to be well organised. An organised approach to cervical screening was introduced in Australia in 1991 the program being funded by the Australian government. Implementation included public recruitment campaigns encouraging all women to screen from the age of 18 through to 70, every two years. Smear takers were educated as to the importance of the sample and that the transformation zone of the cervix needed to be visualised and sampled.

Australia uses conventional Pap smear cytology as its recommended screening test. Extensive quality assurance programs were introduced for laboratories reporting cervical cytology. These include outcome measures of reporting rates, positive predictive values and rates of false negatives. Laboratories are accredited and inspected regularly and failure to comply with quality measures can result in withdrawal of government funding. Formal clinical guidelines for management of screen detected abnormalities have also been implemented. Pap smears are reported using standardised terminology and management recommendations are included on these reports.

A fundamental part of the success of the Australia's cervical screening program has been the introduction of Pap test registers (PTRs). These registers receive information on both cervical cytology and cervical histology on all women entering the screening program. The registers are opt-off and successfully collect over 95% of this data which is used for monitoring the program and for helping deliver quality assurance measures in laboratories. PTRs also help with recruitment and offer failsafe follow-up for women with known disease.

Although Australia's screening program has been successful, one of the main issues remains healthcare of the aboriginal population of Australia. Aboriginal women continue to have a significantly higher incidence of mortality from cervical cancer than the non-indigenous population. The reasons for this are multifactorial and are being addressed.

Australia introduced an HPV vaccination program for boys and girls entering high school in 2007 and 2013 respectively with an uptake rate of over 70%. As a consequence Australia is set to change its Cervical Screening program to use HPV as the Primary Screening test every 5 years from the age of 25 in 2017. Implementation of these changes is underway to ensure a smooth transition continued success of the program and safety of women.

Disclosure of Interest: None declared.

CM04-2

Renewal of the Australian Cervical Screening Program – Compass Trial Leads the Way with HPV Screening

Marion Saville

The Victorian Cytology Service, Australia

The National Cervical Screening Program in Australia has been stable and successful for more than two decades. Nevertheless, the environment in which the program operates has been profoundly disrupted by the introduction of the equally successful National Human Papilloma Virus (HPV) Vaccination Program. The 'Renewal' (or review) of cervical screening is designed to ensure that the success of the screening program continues and that all Australian women, HPV vaccinated and unvaccinated, have access to a cervical screening program that is based on current evidence and best practice. Renewal has involved an assessment of the evidence for the benefits and harms of various screening pathways and a modelled assessment to inform the likely efficacy of the various proposed screening pathways in vaccinated populations. The findings indicated that the effectiveness of the program could be increased, while the expenditure could be decreased, if HPV tests were used in place of cytology. In April 2014, the Medical Services

Advisory Committee recommended that Australia move to a five yearly screening program using an HPV test with partial genotyping for HPV16/18 as the primary screening test, commencing at age 25 and with an exit test between the age of 70 and 74. At a research level, a major trial, Compass, designed to evaluate primary HPV screening in a partially vaccinated population, will generate empirical evidence against which to test the modelled predictions of the Renewal. Together, the evidence review, modelling and ongoing research provide a framework for continuous improvement of the cervical screening program and the potential for further declines in cervical cancer in Australian women.

CM04-3

HPV Vaccination in Australia: Impact on Cervical Screening

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Objectives/Background: Prophylactic vaccines against HPV have been introduced in most developed countries over the last decade. In Australia, a National HPV Vaccination Program for females was implemented in 2007 and young males were included in the program from 2013. Substantial vaccine-induced reductions in infections with vaccine-included HPV types 16/18 and cervical cancer precancerous abnormalities have already been documented in young women. These vaccine-induced changes have driven a major national review of cervical screening, known as 'Renewal'.

Methods: Australia's established organised screening program involves 2-yearly cytology in women 18–20 to 69 years. Although the cytology-based program has reduced cervical cancer incidence and mortality rates by ~50% in the 25 years since its implementation, new modelling for the Renewal evaluation has estimated that further reductions, of the order of 20% or more, can be achieved by moving to primary HPV screening, in women aged 25–74 years with a 5-yearly interval. The relative gains from a move to primary HPV screening will be seen in both older unvaccinated women and also in younger cohorts offered vaccination. In the modelled evaluation, the most effective option for primary HPV screening was found to utilise partial genotyping for HPV types 16/18, with direct referral of this higher risk group to colposcopy. A major Australian trial of primary HPV vs. cytology screening ('Compass'), which is recruiting over 121,000 women in the state of Victoria, is acting as a sentinel experience for the transition of the screening program and is providing early data on the role of primary HPV screening for both unvaccinated and vaccinated women.

Conclusion: Primary HPV screening with partial genotyping for HPV 16/18 allows the cervical screening test to be tailored to detect and manage the same types that the HPV vaccine protects against. In this way, vaccination and screening programs can be tailored to directly complement each other. Australia will transition to primary HPV screening using partial genotyping by May 2017.

Disclosure of Interest: I am co-PI of an investigator-initiated trial of cytology and primary HPV screening in Australia, Compass, which is conducted and funded by the Victorian Cytology Service (VCS), a government-funded health promotion charity. The VCS have received equipment and a funding contribution for the Compass trial from Roche Molecular Systems and Ventana Inc USA. However neither I nor my institution, Cancer Council NSW, on my behalf receives direct funding from industry for this trial or any other project.

CM05-1

Mysteries of Proliferation

Anjula Thomas

Parkway Laboratories, Singapore

Objective: FNAC is an established investigative tool in initial assessment of breast lesions. A correlation study was done to evaluate its overall diagnostic accuracy with emphasis on indeterminate and papillary proliferations.

Methods: Records of all FNA cases from a single surgical unit over a period of 5 years were retrieved. Cytological slides reported as indeterminate, suspicious and malignant were reviewed. Histological correlation was available for all.

Results: There were no false positives. Within the indeterminate categorization which included epithelial proliferation, proliferation with atypia, and papillary or mucinous lesions, 25% were found to be malignant on subsequent histology.

Conclusion: The gray zone tumours inclusive of papillary neoplasms, low grade DCIS and apocrine leisons were found to be the most challenging. These will be discussed with case illustrations.

There has been a shift to core biopsies or mammotome biopsies in recent years. With good clinicopathological correlates, FNAC remains a fast, effective and cheap modality for selecting patients requiring early intervention.

Disclosure of Interest: None declared.

CM05-2

Endoscopic Ultrasound Guided FNA: Deep Seated Mysteries

Min En Nga

National University Hospital, Singapore

Endoscopic ultrasound (EUS) guided FNAs provide access to previously inaccessible, often deep seated structures such as the pancreas, mediastinal and peri-pancreatic lymph nodes or masses and even the adrenal glands. However, pathologists and cytologists need to be mindful of pitfalls that are specific to this method of access, namely, the presence of contaminant gastrointestinal or pancreatic tissue, which can masquerade as lesional tissue. This is of particular importance during rapid on-site evaluation for adequacy of sampling.

In this session, several cases that pose specific diagnostic challenges will be discussed, and relevant practical points regarding

the differential diagnostic approach are highlighted. Pitfalls such as benign contaminant cells and close morphologic mimics will be highlighted and important distinguishing features discussed. The cases discussed include a combination of common, classical entities as well as rarer entities that are less commonly encountered.

The importance of rigorous clinicopathologic correlation, attention to cytomorphology, as well as close communication with clinicians is illustrated through the cases discussed.

Disclosure of Interest: None declared.

CM05-3

Mountains and Molehills in Cytology – Plural Pleural Problems

Angela Chong

Singapore General Hospital and Seng Kang General Hospital, Singapore

Objectives: The discipline of cytology is part science part art. It also requires a great deal of mindful contemplation against a proper history. This presentation will highlight these aspects.

Materials and Methods: Investigation of pulmonary and pleural problems are common place problems presented to cytopathologists around the world. We will illustrate with a number of case studies the problems faced and the solutions used. In the case of pleural cytology, the use of ' atypical cells' is perhaps a panacea for the cytopathologist but creates a mountian of misunderstanding with the clinician. We believe that the approach has changed over the last 10 years and will highlight what we think are the keys for modern practice.

Results and Conclusions: Using case illustrations we will highlight the diagnostic dilemmas and our algorithm.

Disclosure of Interest: None declared.

CM05-4

Tale of Two Tumours – In Head and Neck Cytology

Sangeeta Mantoo

Singapore General Hospital, Singapore

Case Presentations: 1. A 67-year-old gentleman with a previous VATS right upper lung lobectomy for acinar adenocarcinoma in 2011, presented four years later with Horner's syndrome and an enlarged supraclavicular lymph node. There was a large soft tissue mass near surgical sutures, measuring 13 cm x 8.6 cm, on CT Chest with multiple pleura based nodules, and the mass seemed to encase the SVC. Cytological findings from the supraclavicular lymph node aspirate revealed a predominant population of dispersed and focally aggregated large epithelioid cells with eccentrically placed nuclei, dense blue cytoplasm and prominent macronucleoli. Mitotic figures, apoptotic bodies and scattered macrophages were observed. The patient underwent surgical biopsy.

2. A 23-year-old foreign student with a history of ankylosing spondylitis and a previous tumour resected 4 years ago, followed by adjuvant AI and subsequent homeopathic therapy, with modified neck dissection 2 years later, now presented with a left parotid region mass associated with fever, cough and running nose. FNA was performed and revealed a richly cellular aspirate composed of plump spindled cells disposed singly with interspersed tissue fragments of cohesive cells. The cells exhibited round to ovoid or elongated nucleus with granular chromatin bearing a tiny nucleolus, some displaying little cytoplasm while others showing tapered uni- or bipolar, granular cytoplasm. Moderate cytological atypia was noted and mitoses were easily encountered. Rare acinar formation, bare nuclei and apoptotic bodies were observed. The patient underwent wide excision and the intraoperative findings suggested a large 5 x 6 cm mass encasing internal jugular vein and left common carotid artery.

Conclusion: Features were those of malignancy in both cytological specimens. Subsequent surgical biopsies confirmed the suspicion that was raised on cytological diagnoses, and this will be discussed during the presentation with analyses of findings, appropriate diagnostic differentials in each case and literature review.

Disclosure of Interest: None declared.

CM05-5

Concerning a Strange Thyroid Lesion

Felik Paulus

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This is a case of a 52 year old Malay male who presented with hoarseness of voice associated with a left sided neck lump. An MRI scan revealed a 4.2 x 5.5 x 6.6 cm heterogeneous enhancing mass in the left lobe of thyroid with significant mass effect in the form of compression and right tracheal deviation. The left vocal cord is in the paramedian position in keeping with left vocal cord palsy. A fine needle aspiration biopsy of the mass was performed and cytological examination shows a malignant neoplasm consistent with poorly differentiated carcinoma. The patient subsequently underwent total thyroidectomy, laryngectomy and left extended modified radical neck dissection. The cytomorphological and histological features, differential diagnoses and final diagnosis of the left thyroid mass will be presented during the seminar.

Disclosure of Interest: None declared.

CM05-6

Fine Needle Aspiration Cytology of Breast Phyllodes Tumours Revisited

Tan Puay Hoon

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Breast phyllodes tumours are biphasic neoplasms which comprise a benign epithelial component and a spindle cell stromal compartment that drives biological behaviour. They are classified into benign, borderline and malignant categories based predominantly on the stromal characteristics. Preoperative diagnosis can be achieved through fine needle aspiration or core needle biopsy.

In this discussion, cytological characteristics of phyllodes tumours are reviewed. Examples are drawn from the cytology archives of the Department of Pathology, Singapore General Hospital. Differences from the closely related lesion, the fibroadenoma, are highlighted. Recent discoveries in the genomic landscapes of these tumours are briefly elaborated.

Disclosure of Interest: None declared.

CM05-7

The Myths and Mysterious of Cytology

Fernando Schmitt

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Since the period of *Belle Époque*, using a needle to understand the nature of the lesions became an establish method of diagnose and solid part of multidisciplinary team work for a better patient management. In nowadays cytological samples are used for diagnosis, combining the morphological findings with detection of multiple mutations in multiple regions for targeted therapies even in the presence of a few cells in the aspirate. The blend of various methodologies to arrive at a definitive diagnosis is both fascinating and at times challenging, which is part of the intrigue of cytopathology. Among those recent developments in cytology, the idea that you can put a thin needle into a lesion, move it around a few times, smear the cells on a slide, analyze the type of cells, the type of their arrangements, the type of their relations with stroma and make a diagnosis in a few minutes is still appealing in pathology.

In this seminar, interesting FNA cases will be discussed; helping to breaking some myths about the limitation of the method and revealing the mystery and art that combined with science make cytology fascinating.

Disclosure of Interest: None declared.

CM06-1

FNA Cytology of Salivary Gland: Diagnostic Pitfalls

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Salivary gland tumors are uncommon and constitute 2–6.5% of all head and neck neoplasms. FNAC is commonly performed for the management of salivary gland lesions. The main clinical issues are:

(1) distinguishing salivary from non salivary lesions

(2) neoplastic or non-neoplastic

(3) neoplastic lesions: Sub-classification into benign or malignant lesions

(4) proper typing of malignant lesions

1. Distinguishing Salivary and Non Salivary Lesions:

This is important as intraparotid lymph node and submandibular region lymph nodes may be involved by tuberculosis or metastatic carcinoma. Sometimes, paraganglioma may also be present in the same region and are referred to the pathologists as lymphadenopathy or salivary gland tumors. The correct information provided by FNAC of such lesions can modify the management.

Whether Neoplastic or Non-Neoplastic: Non-neoplastic lesions such as cysts, sialadenitis etc can be medically managed and these lesions do not require surgical exploration. Rarely pleomorphic adenoma, adenoid cystic carcinoma and acinic cell carcinoma may yield fluidy aspirates and require thorough screening. The Warthin's tumors have fluidy aspirates but the main clue lies in identification of oncocytes and lymphoid tissue in a dirty background.

In HIV positive patients, there can be a lymphoepithelial lesion which may have epithelial cells and lymphoid cells.

2. Sub-Classification of Salivary Neoplasia:

Solid lesions of salivary gland are rarely misdiagnosed. The cystic lesions are more likely to be underdiagnosed on FNAC as benign.

Majority of the salivary gland tumors require surgical excision.

Pleomorphic Adenoma: This may be misdiagnosed as adenoid cystic carcinoma.

Warthin's Tumor: This lesion should be distinguished from cystic variants of pleomorphic adenoma and mucoepidermoid carcinoma.

Mucoepidermoid Carcinoma: It may be diagnosed as benign lesion such as benign cyst, sialadenitis, Warthin's tumor and pleomorphic adenoma of cystic type.

Adenoid Cystic Carcinoma: Main D/D is pleomorphic adenoma.

Acinic Cell Carcinoma: The main differential diagnostic entities are – adenoid cystic carcinoma, mucoepidermoid carcinoma, and metastatic renal cell carcinoma.

Undifferentiated Carcinoma – is a diagnosis by exclusion.

Malignant Pleomorphic Adenoma: History of long duration salivary gland swelling with recent increase in size helps in suspecting this lesion.

Disclosure of Interest: None declared.

CM06-3

Challenges in the Diagnosis of Lymphoma in Head and Neck Region

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Lymphomas are primary neoplasms arising from lymphoid cells. The purpose of this presentation is to highlight the challenges in their diagnosis in the Head & Neck region by Fine needle aspiration cytology and the utility of employing the most appropriate ancillary technique such as flow cytometry or immunocytochemistry. There are 3 main challenging areas-i. Distinction of primary lymphoma from reactive hyperplasia. ii. Diagnosis of rare forms of lymphadenitis/diseases which mimic lymphoma. iii. Lymphoma vs. Metastatic Carcinoma.

The most common cytological diagnosis of lymphadenopathy in the head and neck region is reactive hyperplasia. Reactive hyperplasia mimics at times a low grade lymphoma. Flow cytometric immunophenotyping is the most suitable ancillary technique in this scenario as demonstration of a monoclonality clinches diagnosis of lymphoma.

Rare forms of lymphadenitis that mimic a lymphoma are: a) Kikuchi-Fujimoto disease or histiocytic necrotizing lymphadenitis affects cervical lymph nodes and smears shows a population of lymphoid cells and histiocytes with extensive karyorrhectic debris/apoptotic bodies in the background mimicking a lymphoma; Kikuchi histiocytes or crescentic histiocytes with/without engulfed nuclear debris are characteristic of this disease; typically there are no neutrophils. b) Castleman's disease can involve cervical nodes which can present as massively lymphadenopathy. Smears show a reactive lymphoid population with an excess of plasma cells. Capillary fragments are also conspicuous and these are surrounded by reactive lymphocytes. c) Rosai-Dorfman disease also involves cervical lymph nodes and shows a reactive population of lymphocytes and histiocytes which typically show emperipolesis, characteristic and diagnostic of this entity. d) Langerhans cell histiocytosis also shows a reactive population of cells comprising variable numbers of lymphocytes, histiocytes, eosinophils and plasma cells. Histiocytes may be mono- or multi nucleated and are characterized by longitudinal nuclear grooves. The above three conditions mimic Hodgkin lymphoma. Careful search must be made for the atypical cells that characterize Hodgkin lymphoma. Role of immunocytochemistry will be discussed.

The diagnosis of metastatic carcinoma is usually straightforward; however challenging situations are: a) metastatic nasopharyngeal carcinoma vs. Hodgkin lymphoma; b) metastatic small blue round cell tumors vs. lymphoma; c) Anaplastic large cell lymphoma vs. poorly differentiated carcinoma; d) metastatic seminoma vs. lymphoma. Subtle morphological features favouring diagnosis of carcinoma or a lymphoma will be discussed along with the importance of applying immunocytochemistry on cell blocks.

Disclosure of Interest: None declared.

CM06-4

Fine Needle Aspiration Cytology of Round Cell Tumors of Head and Neck Region

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Malignant round cell tumors (MRCTs) are a group of heterogeneous tumors that mostly occur in paediatric patients, including young adults. Morphologically, these tumors are characterized by small, round, relatively undifferentiated cells and appear as 'small blue round cells'.

Various MRTs occurring in head and neck region include Ewing sarcoma/PNET, rhabdomyosarcoma, non-Hodgkin's lymphoma, neuroblastoma, poorly differentiated synovial sarcoma, along with rare cases of merkel cell carcinoma and certain adnexal tumors, the latter that can be misdiagnosed as MRCTs. Exact diagnosis of various malignant tumors, including in head and neck sites has definite therapeutic relevance. Ancillary techniques, such as immunocytochemistry or immunohistochemistry, along with molecular techniques are crucial for an exact diagnosis in such cases. Presence of binucleate cells and plasmacytoid, tadpole/racquet shaped cells against a 'lacy' background is suggestive of a rhabdomyosarcoma (RMS). Small round cells with dark nuclei, along with relatively larger cells, exhibiting cohesion, in form of 'rosettes', containing vacuolated cytoplasm and vesicular, fine chromatin, are features suggestive of Ewing sarcoma (ES)/PNET. Presence of dyscohesive malignant round cells with numerous lymphoglandular bodies in the background is suggestive of NHL, in most cases. Presence of round cells with 'speckled' chromatin arranged in a rosetting pattern is suggestive of a neuroblastoma. By immunohistochemistry, CD99/MIC2 and Fli1 positivity with LCA negativity is helpful in confirming a diagnosis of ES/PNET. Desmin positivity with MyoD1 and or myogenin positivity is useful in reinforcing diagnosis of a RMS. Synaptophysin and chromogranin positivity are useful in confirming diagnosis of neuroblastoma. LCA, along with lymphoma specific markers (CD3, CD20) and others are necessary for an exact diagnosis of NHL. In cases of equivocal immunostaining results, in round cell sarcomas, molecular techniques are necessary for resolving diagnostic dilemmas. For example, demonstration of *EWSR1* gene rearrangement for diagnosis of ES. Clinical correlation is imperative in all cases.

This presentation will focus upon various cytomorphological clues that help in judicious application of certain immunohistochemical markers, along with molecular diagnosis in select cases, especially in our settings, for an exact diagnosis of malignant round cell sarcomas, including their rare diagnostic mimics, occurring in head and neck region.

Disclosure of Interest: None declared.

CM06-5

Cytology of Head and Neck Soft Tissue Tumors

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Objective: Fine needle aspiration (FNA) is commonly used to diagnose head and neck mass lesions. Soft tissue neoplasms (STN) are less common than epithelial tumors in this anatomic zone and may be difficult to diagnose especially when unexpectedly encountered. We aim to characterize soft tissue tumors on cytology and compare features on histopathology. In addition a clinicopathologic and immunohistochemical correlation was established.

Materials and Methods: Fifty six FNA specimens from 55 patients with STN of the head and neck were retrospectively analyzed for clinical, cytomorphological and radiological data. FNA was performed by either palpation or radiological-guidance (CT or ultrasound) with on-site evaluation of adequacy by a cytopathologist or cytotechnologist.

Results: Forty two (77%) STNs were found to be primary to the head and neck region while 14 (23%) were metastatic to the head and neck region from distant sites. Twenty five (45%) FNA specimens were of pediatric cases. Twenty one cases had corresponding histopathology records. Immunocytochemistry was performed in 12 cases to establish the diagnosis. The most common diagnosis includes Rhabdomyosarcoma, Ewing sarcoma/PNET and Langerhan cell histiocytosis. Two cases were poorly differentiated carcinomas in corresponding histopathology which were correctly identified on cytology by cytokeratin and P40 immunostains. The sensitivity of FNA in this series was 87.5%.

Conclusion: The head and neck region is involved by a number of primary and metastatic soft tissue sarcomas. Rhabdomyosarcoma and EWS/PNET were the most common soft tissue sarcomas on FNA in the head and neck. There should be a high index of suspicion for poorly differentiated carcinomas or sarcomatoid carcinomas. While not always diagnostic, FNA of STNs can provide an accurate diagnosis with the help of ancillary studies without requiring an excisional biopsy.

Disclosure of Interest: None declared.

CM06-6

Cytology of Skin Appendageal Tumors of Head and Neck

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Workup of patients with cutaneous nodules present a wide range of epithelial and nonepithelial proliferations besides the non-neoplastic lesions. Over last few decades fine needle aspiration cytology has been increasingly utilised for investigation of skin nodules especially to differentiate metastatic from primary soft tissue and skin adnexal tumors (SAT). Even within primary skin adnexal tumors, there is great variation in recognition of nature of adnexal tumor and to exactly subtype the tumor. According to literature, SATs are more common in head and neck region comprising approximately 65% of all appendageal tumors in body. Therefore awareness of cytologic features of these tumors is essential while evaluating a cutaneous nodule of head and neck region. Most of these tumors are benign (around 97%) and malignant tumors are rare, locally aggressive and have a poor outcome. Even among benign tumors also, some might be markers for syndromes associated with internal malignancies.

Therefore, establishing a diagnosis of malignancy and/or associated syndromes in SAT is important for therapeutic and prognostic purposes. However in comparison to head and neck region, malignant tumors are more common in trunk with male predominance. Due to numerous subtypes with the complicated nomenclature, varied histomorphology and the frequency of differentiation along two or more adnexal lines in the same tumor, diagnosing these tumours may be challenging even to an experienced pathologist.

SATs include tumors oriignating from apocrine and eccrine sweat glands, sebaceous glands and hair follicles. They exhibit considerable overlap in clinical and morphological findings. But still it is possible to render a correct diagnosis many times.

Tumors of eccrine glands are proliferations of eccrine ductal or glandular tissue or both in a hyalinized stroma, with lymphocytes as the main inflammatory infiltrate. Even though morphology differs according to subtype of eccrine tumor, usually tightly cohesive epithelial cell clusters with moderate to abundant amount of cytoplasm is seen. Dual cell population is encountered in some categories. Malignancy arising from eccrine glands constitute only a minute fraction of sweat gland neoplasms. Benign tumors like eccrine spiradenomas, acrospiromas, syringomas, cylindromas can undergo malignant transformation. Clinically, the malignant transformation can be suspected by rapid growth of a long standing cutaneous nodule and attainment of large sizes. Cytologically, loss of nested or trabecular pattern of cell distribution, cellular atypia, nuclear pleomorphism and hyperchromasia, increased mitotic rate, necrosis and absence of dual cell population are features suggestive of malignant transformation.

Cytologic smears of apocrine tumors show sheets of discrete large cells, abundant eosinophilic cytoplasm and eccentric, basally located monomorphic round nuclei. The presence of cells with coarsely vacuolated cytoplasm and starry or multillobated nuclei (mulberry cells) within a tumour is indicative of sebaceous differentiation. These can be sebaceous hyperplasia, adenoma and carcinoma. Sebaceous carcinoma can be diagnosed by large pleomorphic cells with hyperchromatic nuclei and one or multiple prominent nucleoli, arranged in clusters and discretely.

Follicular differentiation in adnexal tumours is characterised by the presence of proliferation of basaloid germinative cells, peripheral nuclear palisading and adjacent mesenchymal cells. Follicular differentiation may also be suspected in the presence of matrical shadow (ghost) cells, and/or calcification. Features for malignancy are similar to other malignant adnexal tumors like dyscohesive cells, cellular pleomorphism, nuclear enlargement, irregular nuclear contour, chromatin irregularities, atypical mitotic figures and presence of necrosis.

Many times, it is the only of academic interest to give an exact label to a SAT because most clinicians do not bother about the subtype and pay more attention whether it is benign or malignant and what is the prognosis once it has been removed. Although exact subtyping of tumors of skin adnexa may not always be essential,

certain benign adnexal tumors have aggressive counterparts (e.g., eccrine spiradenomas), and others clinically mimic metastases or small round cell carcinomas (e.g., Merkel cell tumors). Thus, correct cytodiagnoses and awareness of the limitations of cytology (such as in the assessment of local invasion) in these instances help to outline surgical management. Hence, before going for cytologic evaluation, patient's age and sex, whether the lesion is solitary or multiple, location(s) of the lesion, the rate of tumour growth, and, if any associated inherited or systemic diseases are present. Morphological evaluation is very important in evaluating skin adnexal tumours, and special and/or immunohistochemical stains may occasionally serve as ancillary tools.

Disclosure of Interest: None declared.

CM06-8

Enigmatic Thyroid Lesions

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Case 1:

A twenty five year old male presented with midline neck swelling since five months. It was gradually increasing in size. Patient was on tablet Eltroxin 100 mcg since two months for hypothyroidism diagnosed outside.

Examination revealed a midline, firm, non-tender, well-defined neck swelling measuring 3X2 cms. It moved with deglutition but not with the protrusion of the tongue. No palpable neck nodes were seen.

T3, T4 & TSH were within normal limits. Radio-iodine scan showed a cold nodule.

CT Neck: Well defined, poorly enhancing mass in the isthmus of the thyroid gland. There was no lymphadenopathy
Fine needle aspiration cytology of this nodule was request

Case 2:

A thirteen year-old boy presented with painless midline neck swelling for six months duration. There was no history of fever, loss of weight, difficulty in breathing or swallowing. He had a past history of abdominal tuberculosis, two years back, treated with Aanti-tubercular therapy. Examination revealed enlargement of the thyroid gland. It was diffuse, 4x3 cm, irregular & firm in consistency. Systemic examination was normal. Thyroid function test-Normal. All routine blood investigations were normal.

CECT of Neck: An enlarged thyroid gland with heterogenous contrast enhancement and slight tracheal compression. The right lobe measured 2.76 x 2.74 cm and the left lobe measured 2.06 x 2.28 cm in size.

Ultrasound Neck: Enlargement of both lobes and isthmus of the thyroid with a lobulated margin and few well defined hypoechoic nodules.

Fine needle aspiration cytology of the thyroid swelling was performed.

Disclosure of Interest: None declared.

CM06-10

Conjunctival Impression Cytology: An Overview

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Conjunctival Impression cytology (IC) is the technique of collection of the most superficial layers of the ocular surface by applying different collecting devices (usually filter papers), so that cells adherent to that surface are subsequently removed from the tissue. It has become a routine technique to evaluate squamous metaplasia and goblet cell changes in any ocular surface disease, especially in dry eye-related disorders. Clinical diagnosis of dry eyes involves cassette of tests including Tear Breakup time, Rose Bengal, lissamine green, and fluorescein staining are used to evaluate epitheliopathy. Schirmer test, Tear evaporationtest but CIC provides the objective diagnosis of dry eye. Its use as a research tool has experienced an enormous growth and has greatly contributed to the understanding of ocular surface pathology in the last decade.

We did series of studies using CIC over period of 10 years on patients who are at high risk for dry eyes like diabetic patients, soft contact lenses users, patients of glaucoma using β blockers and effects on patients using different tear substitutes like polyvinyl alcohol with povidone, hydroxypropylmethyl cellulose and carboxymethylcellulosewere also seen.

The ability to obtain multiple samples of the ocular surface at one sitting with minimal discomfort to the patient makes it an ideal method of investigating ocular surface disorders when the diagnosis is not clinically obvious or when the clinical diagnosis needs to be substantiated and documented. CIC can therefore detect early quantitative changes of the ocular surface and serves as a prognostic device in the treatment of dry eye. It is being used for diagnostic purposes and to follow the course and efficacy of therapeutic interventions.

Disclosure of Interest: None declared.

CM06-11

Small Cell Variant of Anaplastic Large Cell Lymphoma Presenting as Leukaemia: A Case Report

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Anaplastic large cell lymphoma with a small cell pattern is a rare T-cell lymphoma. This condition is more frequently seen in young patients and should be considered in patients presenting with leucocytosis and constitutional symptoms. We report a case of small cell variant (SCV) of ALCL in a *68 year* old man diagnosed by ALK immunohistochemistry (IHC) and cytogenetic analysis. The limitations of using only morphology in diagnosing this rare variant, is emphasised.

Disclosure of Interest: None declared.

CM06-12

A Case of Clear Cell Sarcoma – An Usual Site of an Unusual Case

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Soft tissue sarcoma also known as melanoma of the soft parts is a rare tumor and was first described by Enzinger in 1965. It is thought to arise from the tendons and aponeurosis. It is known to show a unique genetic rearrangement. This tumor lacks cutaneous invasion and mainly metastases to the lymph nodes and lungs. It arises mainly in the distal extremities of young adults and rarely involves the head and neck. A case of Clear cell Sarcoma occurring in the head and neck region is presented.

Case Details: A 30 year old male presented to the OPD with a gradually increasing painless swelling over the occipital region of one year duration. The swelling measured 5x 4 cms and was firm in consistency. The overlying skin was normal and showed a scar. The patient had undergone surgery for a similar swelling 5 years before details of which were not available. Patient underwent FNAC of the swelling which showed a moderately cellular smear predominantly composed of single dyscohesive and an occasional loose cohesive cluster of round to oval cells with moderate amount of eosinohilic cytoplasm and eccentrically placed nuclei with prominent nucleoli. An occasional binucleate cell was present. Based on the cytomorphological features a diagnosis of Soft tissue sarcoma was offered. The swelling was excised and a final diagnosis of Soft Tissue sacroma was made based on the IHC findings. The patient presented with recurrence and lymph nodes metastasis after about 6 months.

Disclosure of Interest: None declared.

CM06-13

Metastatic Follicular Carcinoma of Thyroid with Occult Primary a Case Report

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Abstract: Papillary Carcinoma is most common type of thyroid malignancy. However in Iodine deficient areas follicular carcinoma is more prevalent making up to 25–40% of thyroid cancers. Follicular Carcinoma comprises approximately 5% of thyroid cancer. Iodine deficiency, older age, female gender & radiation exposure are some of the risk factors for it. Clinically it usually presents as solitary mass in thyroid however occasionally it can present as distant metastatic nodule with often clinically inapparent thyroid lesion. We have reported two cases of follicular carcinoma of thyroid presented as distant metastatic nodule, bone metastasis with clinically occult thyroid nodule. FNA of both the bony nodules well show diagnostic morphology of follicular lesion of thyroid, metastatic origin. During follow up of the patient, FNA of thyroid under imaging guidance show typical cytomorphological features of follicular neoplasm of thyroid.

Conclusion: Follicular Carcinoma has marked propensity for vascular invasion and it disseminates hematogenously and metastasize to bone, lung, brain & liver. Follicular carcinoma avoids lymphatic spread & hence lymph node metastasis exceedingly rare.

Disclosure of Interest: None declared.

CM06-14

Cytology of Head and Neck Lesions, Metastatic Tumors and Unknown Primaries

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Cytology of Head and Neck Lesions encompasses a gamut of lesions and topics that include Salivary Gland Cytology – Diagnostic Pitfalls, Thyroid Cytology – Diagnostic Problems, Challenges in the Diagnosis of Lymphoma and how to approach Lymphomas, Metastatic Tumors, Round Cell Soft Tissue Tumors, Sinonasal Tumors, Skin and Appendageal Tumors, Tumors of the Eyelid and Orbit, Conjunctival impression Cytology an aid in ophthalmic practice and approach to unknown Primaries. Most Metastatic Tumors in the Cervical Lymph Nodes are Squamous Cell Carcinomas of the Head, Neck, Oral Cavity, Upper aerodigestive tract, Lung. Others include Adenocarcinomas of the Stomach, Lung. Rarely Prostatic Cancers can metastasize to cervical lymph node. In young patients metastatic Germ Cell Tumors, Seminomatous and Non Seminomatous Germ cell Tumors must be considered. Small Cell Carcinomas and Neuroendocrine Carcinomas also are included. Moreover High Grade Salivary Gland Carcinomas may be confused with metastatic tumors. A newly described entity NUT Midline Carcinomas must also be considered. In females, Cancer of the Cervix can metastasize to Cervical Lymph Node.

The important ancillary aids are Imaging techniques, like CT, MRI, PET-CT and Tumor Markers. The Differential Diagnosis is High Grade Lymphomas, Malignant Melanomas and Sarcomas.

Disclosure of Interest: None declared.

CM07

Gynecologic Cytopathology: The 2014 Bethesda Nomenclature, Atlas, and Website. An Update and Case-Based Summary

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The 3rd edition of the Bethesda Atlas was published in 2015. This update is the first since 2001 and followed a systematic review of the literature and practice patterns from the prior decade. The new Atlas was increased in subject matter to include new descriptions, illustrations, and cytologic criteria; the integration of special studies; guidelines for clinical management; and additional updated references. In addition to the print version of the Atlas, the entire monograph is available electronically and an even more comprehensive website, the latter administered by the American Society of Cytopathology and which is open to the public.

This workshop will provide a brief overview of the Bethesda process for updates and a tour of the website with important information for users of this valuable international resource. A case-based approach will highlight important updates to the gynecologic cytology terminology as well as the illustration of the basic cytologic criteria for the entities presented in each of the Atlas chapters. In addition, the case-based approach will allow for further discussion of the important look-alikes and mimics of entities which have been added to this edition of the Atlas. Ample time will be available for audience-initiated discussion and questions.

CM08-1

Status of Cervical Cancer Screening in China: Evolution of Testing Methods

Wen Chen, Fanghui Zhao, Qinjing Pan, Xun Zhang, Wenhua Zhang, Youlin Qiao

Cancer Institute/Hospital, Chinese Academy of Medical Sciences, China

Objectives: To introduce the status of cervical cancer screening in China.

Materials and Methods: The performance of VIA/VILI, pap, liquid based cytology, p16/ki67 dual staining, HPV DNA test, HPV mRNA test, E6 oncoprotein HPV mRNA test were evaluated using population based study and case-enriched study.

Results: With the shift of medical strategy from treatment to prevention, two stage of screening program nationwide for cervical cancer were carried out: 10 million rural women were screened using pap between 2009–2012. The second stage screening program covering 30 million rural women is ongoing between 2013–2016. With the increase of workload, the infrastructure of cytology system is unable to bear the burden. The presence of p16/Ki67 dual staining can help improve the sensitivity and efficiency for diagnosis but it is still out of ability, considering the huge population. In 1999, a comparison study was performed to evaluate the efficacy of 6 screening technologies including visual inspection, colposcopy, liquid based cytology, HPV DNA test, etc. Since more and more evidences demonstrated HPV DNA test is more sensitive than cytology, and China has no well-established system for cytology screening, Chinese government decided to nest half million of HPV DNA test in the second stage screening program. In view of the massive demand, various technology platforms were used for HPV nuclear acid detection including real-time PCR, multicolor melting curve analysis, inverse hybridization, Multiplex Luminex® Assays, etc. However, cost, operational complexity and low specificity for HPV DNA test have to be confronted with in the developing country like China. In parallel, Cancer Institute/Hospital collaborated with PATH, QIAGEN and AVC to develop the new technologies: careHPV and E6 oncoprotein tests. careHPV test has been validated as a simple, quick, less cost and portable technology, which passed WHO prequalification, adopt by several less developed countries, e.g. Nicaragua, Uganda, India and Lao. E6 oncoprotein test was demonstrated as a more specific, simple and portable technology, which is a appropriate triage for the high sensitive primary screening method.

Conclusion: China is in the period of the transition from pap/VIA/VILI to more sensitive and specific technologies.

Disclosure of Interest: None declared.

CM08-2

Population-Based Study of DNA Image Cytometry as Screening Method for Cervical Cancer in Rural Areas of China

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Objectives: Worldwide cervical cancer is the third most common cancer among women and over 85% of cervical cancers occur in developing countries. It is estimated that China accounts for 14% of the world's annual incidence of cervical cancer and 12% of the world's annual mortality from cervical cancer. There is no a national screening program for cervical cancer screening in China which remains opportunistic and is based in the cities. 70% of the Chinese population resides in rural areas, where 90% of incident cervical cancer cases might occur. China lacks sufficient cytopathologists, cytotechnicians to interpretate the Pap cytology specimens. The purpose of this study is to compare DNA Image cytometry (DNA ICM) and liquid-based cytology (LBC) as primary screening methods for cervical cancer and precancerous lesions.

Materials and Methods: It was a Wuhan government supported population-based screening program. 15 rural areas in Wuhan were selected. Cervical samples from the women were collected by a brush and placed into fixative solution. Two slides prepared by a Cytospin. One slide was stained by Papanicolaou method for manual cytology examination based on the criteria of TBS, while the other side was stained by Feulgen for DNA ICM analysis. Cervical histologic biopsy was performed if the women with LSIL and above

interpretation in Pap tests and/or 3 abnormal cells with aneuploidy.

Results: In total 181,455 women were screened by using LBC and DNA ICM. The mean age of the women were 39 years ranging from 35 to 45). Compared the results of LBC and DNA ICM, DNA ICM positive rate was increased from 5.4% in women with negative Pap tests to 98.7% in women with HSIL Pap tests. Till now 1498 women had cervical histological follow-up results. Of these women, CIN1+ lesions were diagnosed in 525 cases (35.1%) including 7 cases of invasive cervical cancer (0.47%). The correlation between histological findings and LBC and DNA ICM results is been analyzed.

Conclusion: The preliminary results suggested that DNA-ICM method might be used in those countries where it would be difficult to introduce population based cervical cancer screening due to the lack of cytopathologists and cytotechnologists.

Disclosure of Interest: None declared.

CM08-3

Dual Staining of p16 and Ki-67 in Cervical Smear and Its Potential to Triage ASCUS and LSIL

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Objectives: Detection of simultaneous co-localization of p16 and Ki-67 staining in the same cervical epithelial cell indicates inactivation of pRb in proliferating cells and may lead to HPV related cancerous transformation. Dual staining in cytology may act as an adjunctive biomarker to detect high grade CIN lesions (CIN2 or higher).

Materials and Methods: We studied dual staining of P16 and Ki-67 immunocytochemistry (CINtec®, Roche) in cytology smears of 198 patients of 25 years old or older. All cases had clinical follow-up and biopsy or conization confirmation.

Results: The general sensitivity and specificity of dual staining was 88.4% and 69.6% respectively with a positive protective value of 69.1%, a negative protective value of 88.6% and an accuracy rate of 77.8% to histology confirmation. When stratified by each cytology category, the sensitivity and specificity of dual staining was 66.7% and 66.7% in ASCUS (33 cases), 71.4% and 65.6% in LSIL (46 cases), 90.0% and 60.0% in ASC-H (15 cases), 95.8% and 66.7% in HSIL (51 Cases), and 100% and 75.0% in NILM (58 cases). All 5 cases of CIN2 and higher confirmed by biopsies in NILM were captured by dual staining, indicating its clinical potential to catch high-grade lesions which might be missed by cytology alone.

Conclusion: Application of dual staining in cytologically uncertain cases of ASC-US and LSIL may improve clinical management and triage.

Disclosure of Interest: None declared.

CM08-5

Value of Multiparameter Flow Cytometry Immunophenotyping in the Screening and Diagnosis of T- and NK-Cell Neoplasms on Cytopathology Specimens: Experience in a Chinese Cancer Center

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Objectives: Diagnosis of T- and NK-cell neoplasms using cytology specimens is controversial; especially cytomorphology alone has long been challenged for its reliability within the field. Although increasing English literatures disclosed the worth of flow cytometry immunophenotyping in enhancing diagnostic accuracy of lymphoproliferative diseases of cytology samples, such a cost-effective combination is far away from being recognized in P.R. China. Our study thus aims at investigating the value of multiparameter flow cytometry immunophenotyping (MFCI) in the screening and diagnosis of T- and NK-cell neoplasms on cytology specimens.

Methods: Among 2466 cases of 6-color MFCI performed from June 2010 to Jan. 2016, we carried out a search for cytology cases with optimal specimen adequacy for MFCI, with correlated histology results and clinical confirmation. If any, data of immunohistochemistry and molecular genetic studies were also obtained from the pathology records. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of MFCI for the screening and diagnosis of T- and NK-cell neoplasms were calculated respectively.

Results: 1028 MFCI cases were recruited according to the searching criteria, 10.89% (112/1028) of which were diagnosed or suggested by histology as T- and NK-cell neoplasms, or were clinically diagnosed recurrent malignancies. For screening possible T- and NK-cell neoplasms by MFCI, the sensitivity, specificity, PPV and NPV were 94.64%, 95.63%, 72.60% and 99.32%, respectively. For the diagnosis of T- and NK-cell neoplasms by MFCI, the sensitivity, specificity, PPV and NPV were 68.75%, 98.80%, 87.50% and 96.28%, accordingly.

Conclusions: MFCI can be of great value in the screening and diagnosis of T- and NK-cell neoplasms using cytopathology specimens. However, correlation with cytomorphology, and even molecular genetic studies would be necessary and helpful to overcome its innate limitation.

Disclosure of Interest: None declared.

CM08-6

Lymphoproliferative Disorders in Serous Effusions: Comparison with Histological Assessment

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Objectives: Lymphoproliferative disorders are often complicated by serous effusions, however rare studies have reported the effectiveness of cytology for diagnosing specific subtypes of lymphoproliferative in serous effusions. In the present study, the lymphoproliferative disorders in serous effusions were categorized according to the WHO classification and contrasted with histological diagnosis.

Methods: The serous effusion samples with a final diagnosis of lymphoproliferative disorders between 2004 to 2015 in West China hospital of Sichuan university were collected and reviewed. Cytological diagnosis of serous effusion was compared with histological diagnosis

Results: 210 serous effusion specimens including 163 pleural effusions, 42 peritoneal effusions and 5 pericardial effusions were identified from 181 patients. Based on cytomorphological features and ancillary studies, 77 effusion cases were categorized into specific subtype according to the WHO classification. There were 43 cases of T and NK cell lymphoma, 30 cases of B-cell lymphoma, 3 cases of myeloid tumor and 1 of mast cell sarcoma. Of these serous effusion lymphoproliferative disorders, T cell Lymphoblastic lymphoma/leukemia was the most common subtype. 81 samples from 81 patients had histological assessment. Compared with histological diagnosis, the coincidence rate of cytological diagnosis was 100% in T cell Lymphoblastic lymphoma/leukemias, Plasmacytomas, NK/T-cell lymphomas, myeloid sarcomas and mast cell sarcoma, followed by mantle cell lymphomas (50%).

Conclusion: If cytomorphological features and ancillary studies are combined, it is possible for subclassification of lymphoproliferative disorders in serous effusion, especially for T cell Lymphoblastic lymphoma/leukemia, Plasmacytoma, NK/T-cell lymphoma, myeloid sarcoma, mast cell sarcoma and Mantle cell lymphoma.

Disclosure of Interest: None declared.

CM09-1

Cytopathology in Turkey

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The PAP Test was introduced into Turkey, as in the United States and Europe, in the 1950s. Exfoliative cytology was being routinely practised in many hospitals and private laboratories in the period since 1970s. A few centers also fine needle aspiration (FNA) was introduced even before 1980s. Nowadays performing FNA was became widespread to a large number of pathology laboratory in Turkey.

Foundation of Turkish Society of Cytopathology in 2002 and later on Federation of Pathology Societies played major role in postgraduate education by offering regular scientific meetings, courses on exfoliative & fine needle aspiration cytology. European Congress of Cytology'2011 and six national congresses with international speakers provided nurturing environment for further exchange of ideas not only among (cyto) pathologists but also with burocrats of Cancer Department/Ministry of Health and Higher Education Council. However, there is still a long way to go to achieve the below issues that are discussed at a special meeting by brain storming sessions and by a pre-survey concerning *the second decade of the Turkish Society of Cytopathology*.

Standardization of curriculums and increasing the number of cytopathologists; (10 board-certified and 31 IAC-certificate holder, present).

Implementation of organised screening programs for cancer (cervical, breast etc.) using conventional & new technologies (LBS, molecular, automatization etc.).

Disclosure of Interest: None declared.

CM09-2

Formation and Development of Clinical Cytopathology in the Republic of Kazakhstan

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The 'Clinical Cytopathology' as a speciality had been formed in the Republic of Kazakhstan in the 1960s. First, the method was used in clinical practice during examination of female population during endoscopic studies by exfoliation; later a puncture method was used in hard-to-rich localizations and in operational material taking. Gradually, the demand in this method had grown, and it had been used for diagnosis of oncological and other diseases.

Due to its high efficiency, accuracy and objectivity the cytologic method has become generally available morphological method. The practical and scientific fields had developed, and the appropriate specialists were trained. The Association of Clinical Cytologists of Kazakhstan, established and admitted to the International Academy of Cytology in 1993, played the main role in development of the cytopathology.

The primary goal of the Association is to improve the population's health and increase the effectiveness of examinations by using the clinical cytology method. For achieving this goal it was necessary to create the speciality and train the specialists in the country. That's why one of the objectives of the Association is to enhance the prestige of the cytologist's profession.

The work of the Executive Board of the Association included the training of the specialists in clinical cytology, organizational and scientific activities.

The organizational activity of the Association of Clinical Cytologists of Kazakhstan includes conducting seminars, conferences and publication of methodological recommendations. In total, during its activity the Association held 15 research and practical conferences and workshops, published 17 methodological guidances and educational supplies for practicing doctors. The seminars for cytologists, cytotechnologists and adjacent specialists are conducted annually.

The objectives of the Association are directed at improving the level of specialists by developing the research studies and attracting new technologies, which besides visual assessment of cytologic indices, also includes various structural and functional, morphometric and cytochemical parameters that are necessary in diagnostics, prognosis and evaluation of diseases treatment effectiveness. As a result of the Association members' scientific works, the obtained original data was presented in 15 dissertations for Candidate of Medical Science (M.S.), 2 dissertations for Doctor of Medicine (M.D.) and in numerous other publications.

The Association of Clinical Cytologists of Kazakhstan cooperates with scientists from Japan, USA, Turkey, Russia, Netherlands, Sweden and other countries.

The specialization in clinical cytopathology is particularly relevant due to the government program on screening and introduction of new technologies.

We conduct the trainings in immunocytochemistry, fine-needle aspiration biopsy and liquid-based cytology for the cytologists.

During 23 years of work of the Association of Clinical Cytologists of Kazakhstan, its members are cytologists and cytotechnologists, who are dedicated to the chosen speciality and provide a great assistance in its work.

Disclosure of Interest: None declared.

CM09-3

Cytopathology in Azerbaijan: The Current State and Perspectives

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Pathology service is provided for 96 years in Azerbaijan. The development of cytopathology in our country is the coincidence in the 60–70's of the 20th century. Today, cytopathology service is given in many pathology laboratories of our country. We collected datas on cytopathology service for last one year from pathology departments of 5 large center of our country: Azerbaijan Medical University, National Oncology Center, Central Customs Hospital, Military Hospital of State Border Service and Bureau of Pathological Anatomy. About half part (50%) of all cytology specimens was cervical smear and it was most received specimen in all centers. Fine-needle aspiration (FNA) of thyroid gland (24%) and effusion fluids (16%) were 2nd and 3rd most common specimens, respectively. 10% of all specimens was other specimens (FNA of salivary glands and other head and neck masses, FNA of lymph nodes and breast lesions, urine, exfoliative cytology samples from skin and mucosal surfaces, and others). In recent years, the number of head and neck FNA specimens has increased. The most important reason of this was the establishment of FNA unit in our department (Department of Pathology, Azerbaijan Medical University) in 2009. This unit is still single center in our country where FNA procedures performed by (cyto)pathologist and rapid on-site evaluation used for all cases. Since its inception, more than 500 FNA were performed for palpable head and neck masses. The distribution of cases who have undergone FNA procedure for head and neck masses is as follows: 56% major and minor salivary gland lesions, 18% lymph nodes of head and neck region, 8% intraosseous jaw lesions, 8% other intraoral masses, 7% neck cysts, 3% other head and neck lesions. However, depending on the spreading of kidney transplantation, urine cytology is also actual in our country in recent years as the first-step examination, as well as easy and inexpensive method for screening polyomavirus infection among kidney recipients. About 200 urine samples of kidney recipients were admitted to our department in last year. We think that in our country, in the coming years, the rate of servical smears and thyroid FNA samples will increase. However, specimens such as lymph node and breast FNA, exfoliative smears from ulcerative skin lesions will lose actuality. Hopefully, to train of new and young (cyto)pathologists can help widespread and routine using of rapid on-site examination with FNA procedures.

Disclosure of Interest: None declared.

CM10-1

Cytology and Radiology Collaboration for Thyroid Nodules

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The clinical importance of thyroid nodules comes from the necessity of the exclusion of malignancy, although 95% are benign. And unless proved otherwise, a solitary thyroid nodule should not be assumed as benign.

FNA cytology is considered the gold standard for evaluating thyroid nodules. However, in about 10–30% of the cases, cytology is indeterminate and ultrasonography (US) can provide useful additional information in predicting malignancy for these nodules. Marked hypoechogenicity, irregular margin, microcalcifications, and taller-than-wide shape were considered suspicious features at US. None of these sonographic features has a high positive predictive value for malignancy individually but determine the indication of FNA with clinical findings.

Cyto-sonographic correlation is important to reduce the occurrence of false-negative and false-positive results. The studies in the literature have suggested that if a cytologically benign nodule shows suspicious sonographic findings, repeated FNA cytology should be performed. US features of nodules with suspicious cytological results are useful in planning the extent of surgery. Therefore, the US and FNA cytology are complementary to each other and play a crucial role in diagnosis and clinical management of thyroid nodules. On the other hand, it should be noted that on-site

evaluation during the FNA procedure contributes to maximize the cyto-sonographic correlation.

Disclosure of Interest: None declared.

CM10-2

Follicular Neoplasia/Microfollicular Pattern in Cytology

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Cellular aspirates with scanty amount of colloid composed of microfollicules in >50–70% are evaluated as follicular neoplasia/suspicious for follicular neoplasia (FN/SFN) in thyroid cytology according to the Bethesda System. Microfollicules are composed of less than 15 overlapping thyrocytes forming a circle that is at least two-thirds complete with or without intraluminal colloid. Trabeculae, cell crowding and isolated single cells in the background are other characteristics of these lesions. Microfollicular pattern basically represents adenomatous nodule, follicular adenoma, and follicular carcinoma; however, follicular variant of papillary thyroid carcinoma (FV-PTC), poorly differentiated carcinoma, parathyroid adenoma, and dishormonogenetic goitre are other entities included in the differential diagnosis.

FV-PTC is the biggest trap to be excluded from FN/SFN category. The nuclei of follicular lesions/neoplasms are expected to be round and rather hyperchromatic with coarse granular chromatin, which might be slightly larger than regular thyrocytes. The cases showing nuclear features suspicious for PTC should be reported as 'Suspicious for malignancy' regardless of their prominent microfollicular pattern. However, it is well known that some of the FV-PTCs do not show the characteristic nuclear features of PTC and present with subtle nuclear changes. So, around 15–20% of cases diagnosed as FN/SFN turns out to be FV-PTC at the end. Recently, redefining non-invasive FV-PTC as a neoplasm instead of a carcinoma is discussed in the literature; depending on this approach, the malignancy rate in FN/SFN category is expected to decrease.

Dishormonegenetic goiter presents with microfollicules without intrafollicular colloid; anisokaryosis is another feature; presence of congenital hypothyroidism is the main clue for correct interpretation in these cases. Parathyroid adenomas show a prominent vascular network and neuroendocrine type chromatin. Sheets/clusters or microfollicules may dominate in the aspirates; generally many bare nuclei are present in the background; anisokaryosis and occasionally colloid like-parathyroid secretions can be seen. Particularly for the aspirates from thyroidectomy beds showing microfollicular pattern, parathyroid tissue should always be considered in the differential diagnosis. Poorly differentiated thyroid carcinoma commonly presents with solid/trabecular/insular pattern. Naked nuclei in the background are common; microfollicules may be seen. The cells are characterized by small nuclei with some convolutions, speckled chromatin, and scanty cytoplasm. Mitosis, apoptosis, and rarely necrosis can be seen. Rather uniform small cells in a hypercellular, crowded, partially microfollicular aspirate may cause a misinterpretation as FN/SFN. Nuclear atypia should not be downgraded in these cases.

Disclosure of Interest: None declared.

CM10-3

Oncocytic Lesions/How to Deal With

Sule Canberk

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Oncocytic tumors of the thyroid are uncommon; however, they represent an interesting group of tumors due to their morphologic spectrum, clinical behavior and response to treatment. Fine-needle aspiration (FNA) has proven to be an effective diagnostic tool in the management of oncocytic thyroid nodules. Oncocytic lesions of thyroid present a spectrum from hyperplasia/metaplasia to neoplasia that have always attracted pathologists/cytopathologists for ages by their unique cell morphology. Oncocytic cells have large, finely granular, dense eosinophilic cytoplasm with a round large nuclei and prominent nucleolus. The benign conditions with oncocytic cells are; thyroiditis, Graves' disease, post treatment and post-FNA effects, adenomatous nodules/benign nodules with extensive oncocytic changes. The malignant conditions with oncocytic cells simply divided as true oncocytic neoplasms (ONs) (oncocytic adenoma-OA-, oncocytic carcinoma -OC-) and oncocytic variants of other thyroid malignancies (medullary thyroid carcinoma -MTC-, papillary thyroid carcinoma -PTC-, poorly differentiated thyroid carcinoma-PDC- etc). It is an absolute necessity for cytopathologist to distinguish non-neoplastic oncocytic lesions from the neoplastic counterparts, and define malignancy when it is possible. The question is: 'Does the patient need surgery? The criteria of malignancy, gold standard and the diagnostic challenges for ONs (OA versus OC) are exactly the same as in the follicular adenoma (FA) versus follicular carcinoma (FC). The cytomorphologic criteria of oncocytic neoplasms to some extend helps distinguishing neoplastic lesions from non-neoplastic lesions. Pitfalls in the diagnosis of oncocytic lesions may be analysed in three parts; -the differentiation between non-neoplastic and neoplastic oncocytic lesions, -true oncocytic neoplasia versus oncocytic variant of other thyroid malignancies and – OA versus OC which is mostly impossible by cytology. There are many studies in the literature searching for detailed cytomorphologic clues to make a better diagnostic approach in oncocytic cases. Unfortunately neither demographic studies (size, age predilection etc), nor detailed cytomorphologic analysis provide reasonable help for prediction malignancy for ONs with or without CLT. Multinucleation, enlargement of nuclei, pleomorphism, macronucleoli, grooves, dark-coarse-abnormal chromatin texture often are misinterpreted as atypia in oncocytic aspirates, however, all of them can be seen in both benign and malignant oncocytic lesions.

Briefly, this lecture addresses the value of cytomorphology in the evaluation of oncocytic thyroid lesions concerning the management with a multidisciplinary approach based on the case examples.

Disclosure of Interest: None declared.

CM10-4

Cytopathology of Papillary Thyroid Carcinoma Variants: Diagnostic Issues and Pitfalls

Syed Z. Ali

The Johns Hopkins Hospital, United States

The presentation would highlight the cytomorphology of papillary thyroid carcinoma variants on FNA. Emphasis will be placed on differential diagnosis and achieving higher diagnostic accuracy on cytologic samples. Impact on patient's clinical management, as well as on disease prognosis will be explained. Role of ancillary testing will be briefly discussed.

Disclosure of Interest: None declared.

CM10-5

Mimickers of Thyroid Tumors/Metastases in Thyroid

Aysun Uguz

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Malignant thyroid nodules are fewer than 5% of all thyroid nodules. Fine-needle aspiration (FNA) is considered an first and essential tool in providing a rational approach to the clinical management of thyroid nodules with high sensitivity and specificity especially in malignant cases. However cystic, inflammatory lesions, follicular lesions, and lesions with multinucleated giant cells (MGC) of thyroid can be easily mimicked the malignant thyroid tumors. Oncocytic lesions, papillary carcinoma (PC), and medullary carcinoma (MC) can be potential pitfalls in the evaluation of thyroid aspirations. Cystic processes may cause florid atypia which is a significant cause of false positive and negative results. However, focal nuclear atypia is reported in hyperplastic nodules causing diagnostic difficulties. Pleomorphism, hyperchromasia, elongation of nuclei with occasional grooves and inclusions can be seen in Graves disease and can be evaluated as PC incorrectly.

In inflammatory lesions of thyroid mainly include granulomatous thyroiditis and lymphocytic thyroiditis epithelioid histiocytes can be interpreted as atypical cells and they can be good candidate to be overdiagnosed as PC. MGC are present in a variety of disorders of thyroid. Amyloid goiter and dyshormonogenetic goiter may also mimick the thyroid tumors. Even though metastatic malignancies of thyroid are uncommon, it is always a possibility in the presence of a thyroid nodule, especially in patients with known cancer. Any cytomorphology, which is not typical for common primary thyroid tumours, should alert for this possibility.

Disclosure of Interest: None declared.

CM12-2

How to Maximize the Utilization of the FNA Specimens Toward a More Personalized Medicine

Howard H. Wu

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In the current era, the treatment of an increasing number of malignancies is individually tailored based on tumor typing and molecular profiling. Many advanced cancers are diagnosed solely by FNA, but sometimes even when the direct smears are markedly cellular, the cell blocks lack adequate cellularity for ancillary testing. When this occurs, the cell transfer technique, a simple, cost-effective method for selectively removing tumor cells from FNA direct smears for immunocytochemistry and molecular testing, can be utilized. In this talk, we will present selected cases to illustrate how this technique can maximize the utility of FNA not only for tumor diagnosis but also for necessary molecular testing. The cytomorphologic differential diagnosis for each case will be discussed and the process of selecting the appropriate immunostains and molecular tests will be outlined.

Disclosure of Interest: None declared.

CM12-4

Non-Squamous Lesions in Pap Smears – Cervical Glandular Lesions and Rare Malignancies

Tsui-Lien Mao

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Glandular lesions comprise only a small minority of epithelial neoplasms in Pap smears. However, endocervival glandular lesions are becoming more prevalent in terms of relative proportion and absolute number. Hence, recognition of endocervical glandular lesions, both preneoplastic and malignant, and the differentiation from mimickers, are important and more frequently encountered in our daily practice. Herein, we will discuss briefly the benign and malignant mimickers of endocervical glandular neoplasms, and present several cases of rare non-squamous lesions/malignancies in Pap smears.

Disclosure of Interest: None declared.

CM12-5

Rapid On-Site Cytology Evaluation for EUS-FNA of Pancreatic Tumors: A Single Tertiary Center Experience

Tsu-yao Cheng, I.-shiow Jan, Sow-hsong Kuo

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Endoscopic ultrasound (EUS) has been widely accepted as a good adjunctive tool for evaluating bilio-pancreatic lesions. With the aid of fine-needle aspiration (FNA), EUS has become the most popular method for diagnosing pancreatic tumors. EUS-FNA can offer a highly sensitive and specific cytological diagnosis at rates of 85% and 98% in pancreatic cancer patients. For better quality assurance with improved diagnostic yield, on-site cytology evaluation of EUS-FNA specimens is very useful. The method for rapid assessment of FNA smears is usually rapid Romanowsky-type stain such as Diff-Quik, Hemacolor stain or its equivalent.

Our institution National Taiwan University Hospital has performed EUS-FNA for diagnosing pancreatic tumors since 2001. We adopted on-site cytology evaluation for EUS-FNA specimens with Hemacolor stain in the beginning, and added Ultrafast Papanicolaou (UFP) stain for better assessment of subtle nuclear presentations later. Tumors with distinctive nuclear pattern such as pancreatic endocrine tumor would be easily demonstrated by UFP stain.

On improving skills and methods, we could classify our EUS-FNA practice into first four-year learning period, second four-year experienced period, and subsequent modification period with UFP stain addition. The operating characteristics of EUS-FNA in each period were as follows: sensitivity 93.5%, 95.8%, 96.7%; specificity 100%, 100%, 100%; positive predictive value 100%, 100%, 100%; negative predictive value 82.4%, 90.7%, 90.2%, and accuracy 95.0%, 97.0%, 97.5%.

In summary, UFP stain has offered complementary effects to rapid Romanowsky-type stain with better nuclear features during on-site evaluation for EUS-FNA of pancreatic tumors. Our institution experience has shown combination of UFP stain and rapid Romanowsky-type stain may aid in rapid EUS-FNA cytology diagnosis for pancreatic tumor with good accuracy.

Disclosure of Interest: None declared.

CM12-6

The Importance of Pap Smear in Diagnosing Non-Squamous Malignancies – Emphasis on Endometrial and Extra-Uterine Cancers

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Objectives: The great success of the routine Papanicolaou (Pap) smear in reducing the incidence and mortality of cervical cancers, especially squamous cell carcinoma, has been well acknowledged. Unfortunately, the incidences of endocervical adenocarcinomas, endometrial cancers, and ovarian cancers have paradoxically risen in these years. The purpose of this study is to evaluate the efficacy of Pap smears in detecting cancers other than cervical squamous cell carcinoma.

Materials and Methods: In Taiwan, the national annual Pap smear screening program, for women aged 30 and over, has been launched since 1995. A total of 1520 malignant Pap smear results were identified from the computerized database of the Department of Pathology, Taipei Veterans General Hospital, Taiwan, from January 1995 to December 2014. The clinical and pathological information were obtained from the medical records.

Results: A total of 1520 malignant Pap smears were identified, including 988 squamous cell carcinoma and 532 non-squamous cell malignancies. Non-squamous cell malignancies exceeded squamous cell carcinoma since 2009. The common origin of the non-squamous cell malignancies were cervical adenocarcinoma (37%), extra-uterine (29%) and endometrium (25%). Of them, endometrial and extra-uterine cancers were statistically increased from 2010 to 2014. About 80% of the extra-uterine cancer was originated from the neighboring organs, such as ovary (49%), colon-rectum (19%), and urinary bladder (10%).

Conclusions: Due to the marked reduction of the incidence of cervical squamous cell carcinoma, non-squamous cell malignancies have become the major findings of positive Pap smears especially endometrial and ovarian cancers. At times, Pap smear results were the first indication of these cancers and then initiated appropriate managements. Therefore, the importance of identification of non-squamous cell malignancies in Pap smears cannot be over emphasized.

Disclosure of Interest: None declared.

CM13-1

The Possibilities of Cytological Diagnostics in Breast Carcinoma

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The Purpose of the Study: To show the possibilities of modern cytological diagnosis of breast cancer.

Material and Methods: The analysis of 209 pre-operative Corbiopsy with histological examination of mammary glands and in parallel with smears cytology. Preoperative cytology Fine needle biopsies performed in 384 patients with breast cancer.

In 252 patients with breast cancer compared preoperative and postoperative immunocytochemistry immunohistochemistry in determining the expression of estrogen receptors, progesterone, oncoprotein HER2/neu, a protein proliferative activity of Ki-67.

In 145 breast cancer patients with HER2-status undetermined defined possibility of FISH-studies on cytological material.

Routine cytological preparations stained with azure-eosin dyes. Immunocytochemistry was performed using methods Ultra Vision, EnVision FLEX. Antibody was used firm 'Dako' estrogen receptor (ER), progesterone receptor (PR), oncoprotein HER2/neu, the proliferative activity of the protein Ki-67. Preparations for

the immunohistochemical study was prepared by the method of liquid cytology (Cytospin 4, Thermo Scientiphic Shandon) and stained with the machine Autostainer 360, Thermo Scientiphic Shandon. FISH-reaction to use a set of firm 'Dako', including two-color probe to the gene HER2.

Results of the Study: In parallel histological studies Cor-biopsy and cytology smears match cytological and histological diagnoses was 205 out of 209 cases (98%). In 4 (2%) cases, histological and cytological diagnoses do not match. Preoperative histological study of biopsies Cor-sensitivity was 98%, specificity – 99%, accuracy – 98%, efficiency – 97%, bad stuff –1.4%.

Cytology smears Cor-biopsy sensitivity was 99.3%, specificity – 97.1, accuracy – 98.8% efficiency – 94.3%, bad material – 4.5%.

Preoperative fine needle biopsy followed by cytology sensitivity was 97.9%, specificity 97.6%, accuracy 96.3%, 87.4% efficiency, bad material obtained in 8.6%.

The percentage matches the results of immunocytochemistry and postoperative immunohistochemical study for estrogen and progesterone receptors was 88.6%, for protein proliferative activity of Ki-67 – 86%, for the HER2/neu 93.2%.

In the case of an indefinite immunohistochemical HER2-status conducted FISH-study. The data obtained by amplification of the HER2 gene is fully correlated with the definition oncoprotein using immunocytochemistry.

Conclusion: Fine-needle aspiration biopsy followed by cytology and application of modern methods of immunocytochemistry and FISH-method is an effective preoperative morphological study.

Disclosure of Interest: None declared.

CM13-2

Effectiveness of the Cell Block Technique in the Diagnosis of Metastatic Effusions

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Objectives: To compare the morphological features and cellularity of the Cytospin preparation method with those of the cell block and assess the utility of the cell block preparation method in increasing the sensitivity of cytodiagnosis of malignant fluids.

Materials and Methods: The study was conducted in the cytology section of the National Center of Clinical Morphology.

A total of 51 fluid specimens, 37 women and 14 men, were subjected for diagnostic evaluation for over a period of 13 month.

All samples were examined using cytospin as well as cell block preparation with subsequent immunohistochemistry.

Results: Out of 51 cases, 27 were found to be malignant, in which 18 were pleural fluid and 9 were ascitic fluid. One case was lost for follow-up. 23 cases were reactive.

In 4 cases of malignant effusions cytospins were negative and the diagnosis of malignancy was made using Cell block technique and the primary site was ascertain using immunohistochemistry.

Conclusion: The cell block technique provides high cellularity, better architectural patterns and additional yield of malignant cells. The cell block method is useful for special stains and immunohistochemistry.

Thereby the cell block technique increases the sensitivity of the cytodiagnosis of metastatic effusion as compared with cytospins method.

Disclosure of Interest: None declared.

CM13-3

The Increasing Role of Immunocytochemistry Techniques in Determining the Histogenesis of Primary Tumors and Metastasis in Aspirates and Effusions in Liquid-Based Cytology Specimens

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Objectives: The purpose of this study is to estimate the capabilities of immunocytochemistry (ICC) in the practice of cytopathology to determine the histogenesis of primary tumors and metastases in the material of aspirates and effusions by liquid-based cytology.

Materials and Methods: We retrieved 51 cases of immunocytochemical diagnoses. Fine-needle biopsy specimens were introduced into a 10 ml tube with 2 ml of the balanced salt solution. Preliminary we evaluated the cell suspension by cytospin centrifugation and staining by LEUKODIF 200 (LDF 200). If an adequate cellularity was established, additional unstained cytospin slides were made for ICC. After 5 minute fixation in 5% neutral buffered formalin (NBF) they were air-dried and stained using an immunostainer Bond Max. Effusion specimens were evaluated by cytocentrifugation and subsequent steps were similar to those in case of fine-needle biopsy.

Results: Among 51 cases were 33 women (age, 41÷81, median age 61.45 ± 10.81) and 18 men (age 38÷91, median age 60.56 ± 14.60). Most cases (n = 29) were fine-needle biopsy specimens. The transthoracic needle aspiration was adequate in 92.86% cases. ICC confirmed primary lung cancer with the subclassification in 10 cases. The metastatic origin was confirmed in 3 cases, the suspicion about the histogenesis of primary tumor was made in 1 case. If the differential diagnosis suspected lung and breast cancer a useful diagnostic panel of antibodies: TTF1, cytokeratin (CK)7, CK20, mammaglobin, GCDFP-15, estrogen receptor. Aspirates from lymph nodes revealed histogenesis of primary tumors in 11 cases, the suspicion about the primary site was made in 1 case. ICC of thyroid (n = 3) was inadequate in 1 case of cystic lesion. Cells of metastatic origin were obtained in 1 case. Thyroid cancer was confirmed in 1 case. If both lung and thyroid were differentiated, TTF1, PAX8, and thyroglobulin were useful. Effusions were obtained from 22 patients: pleural fluids (n = 9), peritoneal washes (n = 1), peritoneal fluids (n = 12). ICC was evaluable on 90, 9% specimens. 25% specimens had the benign character. The descriptive report was made in 1 case. The ICC diagnoses had a metastatic nature in 16 cases. Molecular testing for EGFR mutations were

made in 7 cases by cytogenetic laboratory later: preliminary stained (n = 5), or fixed in 5% NBF (n = 2) cytological specimens.

Conclusions: ICC can provide accurate classification for benign/malignant process with subsequent subclassification for primary/metastatic process in the vast majority of cases. Cytology offers a possibility of using either preliminary stained, or fixed in 5% NBF air-dried cytological specimens to molecular testing for EGFR mutations.

Disclosure of Interest: None declared.

CM13-4

Serous Effusions – Past, Present, Future

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Objectives: Serous effusions can be problematic for clinicians and morphologists. Solving the problem of malignancy is often followed by the next questions – primary or metastatic and the location of primary site in case of dissemination. In benign effusions a lot of questions can be raised as well: infections (viral, bacterial, protozoa), cardiac or renal failures etc. For many years cell count, biochemical analyses, stained smears were used to make a final decision. Immunocytochemistry add a lot of useful information for solving some but not all the problems.

Materials and Methods: More than 2000 consecutive cases of pleural and peritoneal effusions from two Moscow hospitals were included to the study. The effectiveness of unstained (native) preparations, conventional smears, cytocentrifuged and liquid-based slides was analyzed. Ancillary laboratory tests (immunocytochemistry, flow cytometry etc.), cell blocks technique, scanned (virtual) slides, microphotographs and morphometry were added in some cases.

Results: 64% of effusions from the patients in S.P. Botkin's hospital and 89% from O.M. Filatov's hospital were benign. In most cases cytology alone gave the necessary information for final decision, but more than 9% of cases needed additional tests and/or consultations. Scanned slides and microphotographs were very useful for final decision, consultations, quality control and teaching as well.

Conclusion: In the era of 'omics' and computerized technologies clinical cytology seems to be very useful for patients with serous effusions, both in rather 'simple' and problematic cases. Clinical cytologist (cytopathologist) can play an important role in personalised healthcare giving the key for individual diagnostic algorithm in difficult cases.

Disclosure of Interest: None declared.

CM13-5

Vision Cyto® Sperm Sediment': Diagnostic Possibilities of Cytology of Urogenital Infections in Sperm Sediment

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Objectives: Recently, the examination of the sediment of the sperm in male patients with poorly defined urogenital discomfort has gained clinical acceptance. Because the classic examination of the urine of patients and their sexual partners often remained inconclusive, frustration in patients and physicians alike are common. Therefore, the microscopic examination of the ejaculate sediment has proven to be critical in establishing a diagnosis. To further improve diagnostic accuracy, a special digital system was developed for the cytological examination of the sample semen sediment and tested in this study.

Materials and Methods: Study participants were 787 men of two clinical hospitals in Moscow/Russia between 2010 and 2015. All men had clinical complaints suggesting urogenital infections. In their female sexual partners an abnormal vaginal flora had been diagnosed. In all of the 787 patients the sediment of the sperm samples were examined by using a newly developed digital system, 'Vision Cyto Sperm Sediment (VCSS)' based Cyto Sperm Sediment (CSS) algorithm. For comparison, the following tests were performed simultaneously on the same samples: microbiological cultures, light microscopy of native and stained specimen (May-Grunwald-Giemsa) and polymerase chain reaction (PCR) of the plasma semen, of prostatic secretions, and of urethral discharge samples.

Result: In 98.2% of patients the sperm sediment disclosed a pathologic microscopic finding such as a species of T.vaginalis, Candida, Gardnerella, Mobiluncus, or other mixed flora, cells with signs of HPV infection, epithelial inclusions morphologically suggestive of chlamydia, foreign bodies or other cells normally not present in sperm sediment. The diagnostic information obtained with other tests applied in the plasma semen, in prostatic secretions, and urethral discharge samples was not proven to be significant. The accuracy of the CSS algorithm was evaluated by calculating the risk indicator values for the same patient derived from several fundamentally different microscopic images. According to the Kornfeld method the risk was 0.6–1.9%.

Conclusion: VCSS is a newly developed unique digital system for increasing the diagnostic accuracy of the microscopic examination of sperm sediment samples; it has a risk indicator value of less than 2%. This value is sufficient for a reliable initial diagnosis for research results. In questionable cases, the presumptive diagnosis has to be confirmed or excluded by additional testing.

Disclosure of Interest: None declared.

CM13-6

Optimization of the Cytological Diagnostics by the Liquid-Based Cytology, Cell Block Technique, and Immunocytochemistry in the Thyroid Cytopathology

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The overall incidence of malignant neoplasms per 100,000 population in Russia in 2014 amounted to 388.9 cases, which is 18.6% above the 2004. Thyroid tumors account for 4.3% of all malignant neoplasms registered in Russia. The number of those patients per 100,000 population has been steadily increasing from 58.6 in 2004 to 97.1 in 2014. At the same time diagnosis of thyroid cancer in the later stages (III-IV) in 2014 is 23.4%, as published in the 'Status of oncological care for the population of Russia in 2014', ed. A.D. Caprin, V.V. Starinskiy, G.V. Petrova, Moscow P.A. Hertzen Research Oncological Institute, 2015 (in Russian).

Objectives: To optimize the cytological diagnostics through the use of the liquid-based cytology (LBC), cell block, and immunocytochemistry (ICC).

Materials and Methods: In 2014, we studied the conventional fine needle aspiration (FNA) samples from 236 patients with thyroid diseases. Of these, for more accurate diagnosis by ICC for 51, FNA was conducted additionally by taking the material into the preservative liquid (LBC-method). Then cell block or monolayer slides (depending on the amount of the resulting FNA material) were manufactured. Reagents by the Dako company: ICC markers of proliferation, markers of apoptosis, markers of malignancy, and others were used.

Result: ICC was performed for a total of 51 patients, for each at least 4 different markers were used. From them: unsatisfactory for evaluation material – 6 patients (11.8%), cell blocks were made – 14 patients (27.5%). ICC for 29 patients (64%) has affected the previous cytological conclusion made by FNA conventional smear: clarification of the type of malignancy – 4; confirmation of cytological diagnosis of anaplastic carcinoma – 1; atypia of undetermined significance – 1 – was changed to a benign process, more probable – an autoimmune thyroiditis; suspicious for a follicular neoplasm was changed to benign process – 5; the cytological conclusion of the benign process has prevailed – 16; differentiated thyroid gland from parathyroid – 2.

Conclusion: 1) More informative is a cytological study of materials, when they are presented in two forms – in the form of conventional smears and in the form of LBC. 2) The manufacture of the cell blocks is preferable to the preparation of monolayer slides (high cellularity, architectonic review, the maximum disclosure of nuclea antigen epitope in the preparation of microscopic sections). It is, therefore, important to produce a material with FNA in an amount sufficient for making cell block. 3) ICC helps to improve the cytological diagnostics in more than 50% of cases.

Disclosure of Interest: None declared.

CM14-2

Rose in a Lung Hospital: 23 Years of Experience (1993–2016) – A Gold Standard?

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Introduction: The most chest clinics in Germany have not an own pathologist/cytologist on site (online survey 2016). In our hospital we have over more than twenty years an cytology laboratory. Head of the cytologic laboratory is the author, an pneumologist and medical doctor in internal medicine. Analysis of the literature show that up to 32% (average of about 20%) of FNA's are nondiagnostic due to scant cellularity or poor preparation. In our practice before the biopsy procedure is completed, the clinical cytologist performs a microscopic evaluation and assesses the adequacy of the specimes. Helpful in our method is the immediate vicinity of laboratory and endoscopy.

Material and Methods: All ROSE-biopsies in the last year (2015) were analyzed for representativity.

Results/Table:

Material (n = 223)	Representativity by maximal 4 needle passes
TBNA (n = 12)	12/12 = 100%
EBUS-TBNA (n = 121)	119/121 = 98.3%
EUS-FNP (n = 40)	39/40 = 97.5%
FNP LK peripher (n = 19)	19/19 = 100%
FNP transthorakal (n = 20)	20/20 = 100%
Imprint Core biopsy (n = 11)	10/11 = 90.9%

Conclusions: 1) ROSE is in the hands of pneumologists with cytologic training an suffizient tool. 2) Cytological training during consult-education in pulmonology is very important. 3) An education program for clinical cytology is initiated by Dr. Heine and the author.

Disclosure of Interest: None declared.

CM14-3

Endosonography-Guided Fine-Needle Aspiration Biopsy: Factors of Influence on the Diagnostic Outcome in Lung Cancer and Pancreatic Tumors

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Objectives: Endosonography-guided fine-needle biopsy (EUS-FNB) has gained wide acceptance as an important, minimally invasive diagnostic tool in pulmonology and gastroenterology. EUS-FNB is restricted only to patients in whom the cytological results may be expected to change the course of management. There is commonly in lung cancer as well as in pancreatic tumors a wide range of results achieved using EUS-FNB. In order to improve re-

sults it seems necessary to find out the factors of influence on the diagnostic process.

Materials and Methods: Between 2005–2012 867 EUS-FNB were performed on 364 patients with resected thoracic lesions of the LungenClinic Grosshansdorf and 307 EUS-FNB on 307 patients with pancreatic tumors of the Asklepios Klinik Barmbek and Asklepios Klinik Altona. Cytological results were retrospectively compared with all following available clinical and histological diagnosis.

Results: Despite of all existing differences between thoracic and pancreatic lesions there were similar results of EUS-FNB regarding sensitivity, specificity and positive predictive value (58.3%, 99.2%, 92.5% vs. 59.4%, 98.5%, 98.1%). Factors of influence on the diagnostic outcome are the sampling of tumor suspicious findings (10 cases each) and the aspiration of inadaequate material (33 of 127 lung- and 49 of 170 pancreatic cancer).

Conclusions: In spite of high specificity and positive predictive values using EUS-FNB the sensitivity level seems to be comparable low. The diagnostic outcome of EUS-FNB could be elevated in lung cancer as well as in pancreatic tumors if aspiration of inadaequate material could be avoided.

Disclosure of Interest: None declared.

CM14-4

A Case of Pancreatic Mass – Can Technology Make It Without Morphology?

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Case Presentation: A 48-year-old female patient presented with a several-week history of upper abdominal pain and a weight loss of 3 kg in two months. Physical examination revealed mildly tender upper abdomen and was otherwise unremarkable. The laboratory findings were also unremarkable, except for elevated serum C-reactive protein (CRP). Both computed tomography of the abdomen and the endosonographic examination revealed a pancreatic head lesion with central necrosis, suspected of being a neoplasm. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) and biopsy of the lesion, as well as peripancreatic lymph nodes, were performed. Histological examination revealed hemorrhagic material and inflammatory cells in the lymph nodes. Cytological examination of the fine-needle lesion aspirate showed cell debris and abundant granulocytic infiltrate without preserved tumor cells. Both procedures were repeated after one week. The histological findings in the pancreatic mass corresponded to those of a necrotizing pancreatitis, again without vital tumor elements. Repeated EUS-FNA revealed the same cytological findings as the previous one with additional rare epitheloid cells, which raised suspicion of a specific inflammation. The consequent molecular pathological analysis highlighted the presence of Mycobacterium tuberculosis.

Objectives: Not applicable.

Materials and Methods: Not applicable.

Results: Not applicable.

Conclusions: Tuberculosis of the pancreas should be considered in the differential diagnosis of patients presenting with an unclear pancreatic mass. Although modern technology keeps opening the way for novel diagnostic and therapeutical approaches, the value of morphology as fundamental diagnostic tool and basis for further decisions should not be underestimated.

Disclosure of Interest: None declared.

CM14-5

Cytomorphology, Next Generation Sequencing, and Fish in Lung Cancer – The Cologne Experience

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Objectives: The Network Genomic Medicine Lung Cancer provides molecular diagnostics for patients with lung cancer. In Cologne a molecular diagnostic platform covering all known driver mutations has been set up for this purpose. A comprehensive analysis of biomarkers is done by combining morphology, Next Generation Sequencing (NGS), other sequencing techniques, and fluorescence in situ hybridization (FISH).

Materials and Methods: We analyzed a series of 310 consecutive cases, in which molecular assays for lung cancer diagnostics were done on cytological material covering a two years period (2014–2015) by retrospective analysis of laboratory data in correlation with review of glass slides in selected cases.

Results: The samples were obtained from more than 30 different institutions from all over Germany. The series includes a wide variety of material: transbronchial needle aspiration (TBNA), bronchial brushing or washing, pleural and pericardial effusion, ascites, transesophageal fine needle aspiration (FNA), and others. The fluid samples were prepared by different techniques: sediment smear, cytospin, cell block, liquid based cytology (LBC). Staining protocols include hematoxylin & eosin (HE), May-Gruenwald-Giemsa (MGG), Papanicolaou, and different immuno stains.

A panel approach for NGS with analysis of several genes and a set of FISH assays were used in each case of pulmonary adenocarcinoma, if sufficient material was available. In squamous cell carcinoma and in small cell lung cancer only FISH analysis of *FGFR1* was done. In the second year additional 'Fast Track Analysis' was implemented for selected codons of *EGFR* and *KRAS*.

Of 240 cases of pulmonary adenocarcinoma analyzed by NGS, six cases were not adequate for evaluation. Wild type sequences for the analyzed genes were found in 30 cases. Genetic changes were found in 204 cases including 45 mutations of *EGFR*, 83 mutations of *KRAS*, 17 mutations of *BRAF*, 16 mutations of *MET*, and 115 mutations of *TP53*. In 104 cases two or more mutations were found in the same sample.

'Fast Track Analysis' was done in 128 cases. In 45 cases the amount of extracted DNA was too low for evaluation. In the remaining 83 cases, wild type sequences were found for the analyzed codons in 48 cases and genetic changes in 35 cases: 26 mutations of *KRAS*, 9 mutations of *EGFR*.

Conclusion: All molecular assays routinely used in our institution for lung cancer diagnostics can be applied to cytological samples. The procedures work well with a wide variety of samples obtained from different institutions and prepared and stained following different protocols. 'Fast Track Analysis' is not available for all cytological samples due to the need for larger DNA amounts.

Disclosure of Interest: Remuneration fees for lectures on lung cytology from Lilly in the year 2012 and from Roche in the year 2013.

CM15-1

Fine Needle Aspiration of Cystic Squamous Lesions of the Head and Neck

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Objectives: A wide variety of squamous cysts occur in the head and neck including several benign developmental cysts (branchial cleft, thyroglossal duct, bronchogenic), cutaneous squamous cysts such as pilomatrixoma, and metastatic squamous cell carcinomas including those associated with high-risk HPV. Aspiration of these squamous cysts can create a variety of challenges.

Materials and Methods: This session will review cytologic features and pitfalls in the diagnosis of cystic squamous lesions of the head and neck with particular emphasis on the FNA and testing of HPV-related squamous cell carcinoma.

Results: Benign developmental squamous cysts of the head and neck such as branchial cleft cysts are more common among younger individuals, and typically consist of bland squamous cells including many anucleate forms in a background of debris and inflammation. Diagnostic problems arise when there are atypical features. In contrast, metastatic cystic squamous cell carcinomas occur primarily in adult patients over 35 years old. HPV-related cystic squamous cell carcinoma of the head and neck is a significant cause of cystic squamous lesions in middle age male adults with a history of exposure to high-risk HPV. A major consideration when encountering these lesions by FNA is how to test for HPV. Various testing options are available including p16 immunochemistry, in situ hybridization (DNA and mRNA), PCR, and various liquid-based technologies.

Conclusions: Correlation of cytological findings with clinical features is important in the assessment of squamous cysts of the head and neck. In addition, testing for high risk HPV is an essential element in the workup of malignant squamous lesions.

Disclosure of Interest: None declared.

CM15-3

The Salivary Gland

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Cystic lesions are estimated to account for up to 8% of all salivary gland masses. They represent a wide range of salivary gland pathology, ranging from the non-neoplastic mucocele, salivary duct cyst and sclerosing polycystic adenosis, to benign tumors such as Warthin tumor and cystadenoma, to several malignant cystic tumors, including low-grade mucoepidermoid carcinoma and acinic cell carcinoma. Occasionally, cystic degeneration also occurs in usually non cystic tumors such as pleomorphic adenoma and basal cell adenoma. In the parotid gland, cystic lesions also include the differential diagnosis of metastatic cystic squamous cell carcinoma to intraparotidal lymph nodes and lymphoepithelial cysts. One of the most commonly encountered diagnostic problems in cystic lesions of the salivary glands is the cytologic distinction between mucocele and low-grade mucoepidermoid carcinoma.

This part of the slide seminar will illustrate a difficult case of cystic and inflammatory salivary gland lesion with histological follow-up, and will focus foremost upon this common diagnostic problem, followed by examination of other differential diagnostic entities and potential pitfalls. Finally, the role of ancillary studies in this setting will be briefly discussed.

Disclosure of Interest: None declared.

CM15-4

Fine Needle Aspiration of Cystic Metastases to Head and Neck

Sule Canberk

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Metastatic tumors of head and neck region are classified as 'metastatic cervical carcinoma with and unknown primary tumor' (MCCUP) and 'secondary tumors to the neck (other than carcinoma from head and neck region)'.

Regardless being primary or secondary, these metastases mostly present as solid and firm lesions, however, a distinct subset of metastatic lymph nodes present as cystic lesions. Detecting malignancy in head and neck region is quiet challenging for a variety of reasons. Particularly, due to the complex anatomy and high frequency of anatomic variations, head and neck region has many limitations for imaging and other diagnostic modalities. Although imaging modalities such as CT, MRI and PET/CT may suggest malignancy, in the absence of clinically known/proven malignancy, the diagnosis is always difficult.

Fine needle aspiration (FNA) biopsy is the primary diagnostic method for head and neck lesions. Sensitivity of the FNA drops to 50–73% from 90–97% in cystic malignancies when compared to

the solid metastases. However FNA is still the best sampling method in a lesion, which is partly or completely cystic and surrounded by a thin solid rim.

The cellularity of the aspirations from cystic lesions is always limited despite the lesion is benign or malignant. Interpreting a hypocellular aspirate is a battle for cytopathologist due to the dilutional effect of the cyst fluid. Even in the presence of adequate cellularity, this region has many benign and malignant entities that may present with overlapping cytomorphologic features. One should be very careful for the possible scenarios when evaluating FNAs from head and neck region that may cause dramatic consequences in patient management.

This presentation mainly focuses on possible scenarios based on case examples of primary non-squamous and secondary cystic metastasis of head and neck region with the detailed cytomorphologic analyses and diagnostic algorithm in a multidisciplinary approach.

Disclosure of Interest: None declared.

CM15-5

Differential Diagnosis of Malignant Cystic Thyroid Lesion

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Thyroid nodules with cystic component account for about one-third of all palpable thyroid lesions. The prevalence of sonographic cystic thyroid lesion, palpable and non-palpable, is about 50%.

The cystic nodules were not different with solid nodules in patient demographics, the rate of solitary lesion, or the nodule size.

Indeterminate cytology demonstrated malignancy with about half the frequency in cystic lesions as compared with solid nodules.

Considerable but much less predictable than FNA results were signs of local compression or invasion, a history of head or neck irradiation, cyst recurrence after aspiration, or an increase in the cystic nodule's size.

The amount and macroscopic appearance of aspirated cystic fluid (clear or bloody) is not predictable of its benign or malignant lesion.

Cytological examination of specimens arising from these cystic nodules may show clusters of follicular cells that allow us to make a cytological diagnosis of benign or malignant nodules. But, the smears only show cystic changes (macrophages), bloody material or cellular absence, especially when cystic component is greater. In these cases a cytological diagnosis of unsatisfactory or nondiagnostic sample is usually made.

A report suggested that nodules with nondiagnostic FNA showed typical cystic changes had a slightly higher rate of malignancy than nondiagnostic FNA showed acellular specimens or only bloody content, which may be in relation with younger age and higher prevalence of lymphocytic thyroiditis in this group of patients.

A report issued that if you meet the cystic lesions also contain few atypical cyst-lining cells which have characteristic features and lacked nuclear crowding, intranuclear pseudoinclusions, and papillary architecture that, in many specimens, allowed them to be recognized as benign. The report recommended that the subset of cells with the characteristic features described in that study be reported as 'consistent with benign cyst lining cells'.

Recent studies suggest BRAFV600E mutation could be a predictable for malignancy. A report suggested that polygonal eosinophilic (plump) cells, microfollicles, intranuclear pseudoinclusions, sickle cells, and cystic changes were significantly associated with the BRAFV600E mutation. Therefore, additional BRAFV600E mutation test could be useful for diagnosis of cystic lesion.

Disclosure of Interest: None declared.

CM16-1

Clinical and Radiological Evaluation of Thyroid Nodules

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Objectives: The diagnosis of thyroid nodules is rapidly increasing in developed countries mainly due to the widespread use of ultrasound. The aim of this presentation is to discuss how clinical information and radiological features can be integrated to help us individualize management.

Materials and Methods: The current evidence on the role and limitations of clinical and sonographic features in the evaluation of thyroid nodules will be summarized.

Results: It has been known for years that specific clinical or radiological features of thyroid nodules are associated with higher or lower risk of malignancy. These features are usually taken into account to guide the need for biopsy. Nonetheless, once the biopsy is performed, we mainly rely on the cytological interpretation to dictate the next management step. Several studies in recent years have shown that the risk of malignancy of a given cytological diagnosis is impacted by clinical and radiological features and therefore should be integrated to individualize management.

Conclusion: Clinical and radiological features have a direct impact on the risk assessment of thyroid cytology and should be integrated to individualize management.

Disclosure of Interest: None declared.

CM16-2

Implications of the Bethesda System for Reporting Thyroid Cytopathology for the Management of Thyroid Nodules

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Objectives: The Bethesda system for reporting thyroid cytopathology has homogenized the terminology around the world, facilitating the interactions between pathologists and clinicians. The impact of the Bethesda system on the management of thyroid nodules 7 years after its publication will be discussed in this presentation.

Materials and Methods: The current evidence on the role and limitations of the Bethesda system for reporting thyroid cytopathology for the management of thyroid nodules will be summarized.

Results: The relatively undefined criteria of the indeterminate categories, and especially of the atypia/follicular-lesion of undetermined significance (Bethesda category III), has led to wide differences in the risk of malignancy (ROM) of these categories among institutions. While this had little interest when the Bethesda system was published, it is of tremendous importance with the advent of molecular markers, because the ROM within a category impacts the sensitivity and specificity of the molecular markers. Several studies have proposed the need for a subclassification of the indeterminate thyroid nodules to improve the assessment of the ROM. Furthermore, the reclassification of the encapsulated non-invasive follicular variant of papillary thyroid carcinoma (ENI-FVPTC) as a benign lesion, as recently proposed, may substantially change the ROM for each of the Bethesda categories.

Conclusions: The diagnostic criteria of the indeterminate categories of the Bethesda system need to be better defined and perhaps further subdivided into subcategories. The ROM of the Bethesda categories needs to be reissued if ENI-FVPTC is no longer considered a malignant diagnosis.

Disclosure of Interest: None declared.

CM16-3

Assessment of Commercially Available Molecular Tests in the Evaluation of Thyroid Nodules

Bryan McIver

Moffitt Cancer Center, United States

Objectives: One fourth to one fifth of the thyroid nodule biopsies render an indeterminate cytological diagnosis. Several molecular marker tests have been recently developed to improve the presurgical diagnosis of these nodules and are already in widespread use in the US. The current evidence, areas of uncertainty, possible applications and actual impact on clinical management will be discussed in this presentation.

Materials and Methods: The current evidence on the role and limitations of the molecular markers for the management of thyroid nodules will be summarized.

Results: The performance of molecular marker tests is strictly dependent on the pretest risk of malignancy, which is influenced by the specific clinical, radiological and cytological characteristics that are also subject to institutional differences. For this reason, the interpretation of molecular marker results should be individualized. Furthermore, the cost-effectiveness and the reliability of the results might be severely compromised in some scenarios. Until larger, independent, validation studies define the clinical impact of these tests, their results should be interpreted with caution.

Conclusions: Molecular markers results need to be individualized and interpreted cautiously as the long term impact on management, patient health, and cost are largely unknown.

Disclosure of Interest: None declared.

CM16-4

Integrating Information in Real Time: The Same-Day Thyroid Nodule Clinic

Kristen Otto

Department of Head and Neck and Endocrine Oncology, Moffitt Cancer Center, United States

Objectives: Any diagnostic process is accompanied by uncertainty that can cause severe stress and anxiety to the patient, especially when cancer is in the differential diagnosis or surgery among the possible treatment options. For these reason, shortening the wait time to diagnosis is desirable. The model of our institutional same-day thyroid nodule clinic will be discussed in this presentation.

Materials and Methods: The organization, sequence of events, and results of our same-day thyroid nodule clinic will be detailed.

Results: Shortening the time to diagnosis significantly reduces the stress and anxiety; increases patient satisfaction; and may improve outcomes of aggressive cancers. To achieve a same-day diagnosis requires multidisciplinary interaction to appropriately integrate information and individualize management.

Conclusions: A same-day thyroid nodule clinic is feasible, convenient and desirable.

Disclosure of Interest: None declared.

CM16-5

New Technologies and Future Directions for the Assessment of Thyroid Nodules

Anthony Magliocco

Morsani College of Medicine, University of South Florida Health Science Center, United States

Objectives: There are numerous new diagnostic laboratory technologies emerging that will likely impact the analysis of thyroid nodules in the future. These include advances in molecular diagnostics and analytical microscopy methods. Analytical mi-

croscopy provides opportunities to standardize measurements of cellular components and potentially improve diagnostic accuracy.

Materials and Methods: Analytical digital microscopy methods will be discussed.

Results: Recent advances in digital image analysis open new opportunities to improve the precision and diagnostic accuracy of pathology diagnosis. New advances in reagent systems and digital analysis will also enable more functional information regarding signaling pathways in tissue to be determined.

Conclusions: Advances in digital microscopy and functional tissue analysis will impact the practice of cytopathology in the future.

Disclosure of Interest: None declared.

Cytotechnologist Session

CT-1

A Hoop, Three Bridges and a Path

Celestino Rodrigues Pereira

Department of Pathology Kantonspital Aarau Switzerland, Switzerland

My job involves a great deal of travel to developing counties in order to educate and screen the local population for breast and cervical cancer. In order to do that, I would like to speak on the topic in three sections. First, my job initially involves spending a day or more personally training the local physicians, nurses, technicians and health care administrators on how to screen for these types of cancers. Once this phase is complete, the public education phase is the second phase. I normally address several hundred local citizens on the basics of cancer and the need for screening. My goal in this phase is to educate and persuade as many people as possible to come to a clinic for this type of screening. Finally, the team I have trained and I are engaged in preparing and analyzing the results of pap smears and other procedures. Each of these three phases has its particular challenges.

Disclosure of Interest: None declared.

CT-2

The Evaluation of Washing Cytology from Ureter and Renal Pelvis: Presentation of a Newly-Devised Washing Fluid

Yuuji Aoki, Shiho Azami, Mizuki Iino, Azumi Sakaguchi, Kanako Ogra, Toshiharu Matsumoto

Department of Diagnostic Pathology, Juntendo University Nerima Hospital, Japan

Objectives: The washing cytology from ureter and renal pelvis is a useful method for diagnosis of the localization and spreading of the tumor. However, the obtained cells by the washing cytology were degenerated by the washing of physiological saline. Therefore, it is difficult to observe the cell morphology, and thus there were a lot of cases with the difficulty for the differentiation of benign or malignancy on the microscopic examination. In the current study, we report the results of the evaluation of cell morphology using infusion preparation and physiological saline, and we decide a most suitable washing fluid for the washing cytology of ureter and renal pelvis.

Materials and Methods: The operated materials of ureter and renal pelvis, lymph nodes having urothelial carcinoma were washed by physiological saline and infusion preparation (an acetic acid Ringer's solution and a lactic acid Ringer's solution), thereafter cytological diagnosing specimens were made from a suspension. In the cytological diagnosing specimens, the cell morphology including nuclear findings, cytoplasm findings, and the size of nuclear area were evaluated in each of the washing fluids.

Result: In the physiological saline, nuclear chromatin structure became indistinct, and the nuclear area was large. The cell degeneration was remarkable, and the cell morphological observation was difficult. In the acetic acid Ringer's solution, nuclear chromatin structure was clear, and there was little degeneration, and the cell morphological observation was easy. In a lactic acid Ringer's solution, the nuclear chromatin structure showed concentration form, and the nuclear area was small.

The cell degeneration was remarkable, and the cell morphological observation was difficult.

Conclusion: The comparison of washing fluids led to the conclusion that the acetic acid Ringer's solution is the best washing fluid for the washing cytology of urothelial carcinoma of ureter and renal pelvis.

Disclosure of Interest: None declared.

CT-3

Cytologic Features of Follicular Thyroid Neoplasm Differentiating from Its Mimicking Lesions

Hwa-jeong Ha, Jung-soon Kim, Myung-soon Shin, Woo-tack Song, Jae-kyung Myung, Hye-sil Seol, Sun-hoo Park, Jae-soo Koh, Seung-sook Lee

Department of Pathology, Korea Cancer Center Hospital, Kirams, Korea

Objectives: Fine needle aspiration cytology (FNAC) is a useful tool in the evaluation of thyroid lesions. However, it is difficult to make a correct diagnosis of follicular neoplasm (FN) in FNAC because of overlapping cytological features with other mimicking lesions. The aim of this study is to elucidate the cytological features of FN by comparing with cytologically mimicking lesions.

Materials and Methods: One hundred and sixteen cases of thyroid FNAC specimens diagnosed as FN which have their final histological diagnosis were included in this study.

Results: Final histological diagnoses of the 116 cases were variable, including 27 follicular adenoma (FA) (23%), 23 follicular carcinoma (FC) (20%), 35 follicular variant of papillary carcinoma (FVPTC) (30%), 12 classic papillary carcinoma (PTC) (10%), 17 cellular nodular hyperplasia (NH) (15%), one Hurthle cell carcinoma (1%), and one Hashimoto's thyroiditis (1%). We evaluated the cytological architectural patterns, nuclear features, and colloid according to histological groups. Microfollicular pattern was observed in 100% of FN and 83~100% of remaining lesions. Multilayered rosette was observed in 24% of FN and 0~46% of remaining lesions. Branching monolayered sheets were found in 8% of FN and 0~18% of remaining lesions. Syncytial fragments were observed in 96% of FN and 0~100% of remaining lesions. Trabecular pattern was noticed in 34% of FN, but it was not observed in the remaining lesions. Chromatin clearing was found in 10% of FN and 0~86% of remaining lesions. Nuclear grooves were observed in 32% of FN and 0~100% of remaining lesions. Intranuclear inclusion was noticed in 10% of FN and 0~51% of remaining lesions. Anisonucleosis was appreciated in 54% of FN and 0~100% of remaining lesions. Colloid was found in 66% of FTN and 0~100% of remaining lesions.

Conclusions: These study shows that microfollicular architecture is very common among FN, NH, and FVPTC, but trabecular pattern was exclusively present in FN. Additionally, while it is not always easy to find intranuclear inclusions or frequent nuclear grooves in FVPTC, nuclear clearing and/or relative nuclear anisonucleosis still can be differentiating features from FN.

Disclosure of Interest: None declared.

CT-4

Co-Operative Study between MNUMS and Gunma University on Cyto-Pathological Practice and Education

Tomomi Yoshida, Toshio Fukuda

Department of Laboratory Sciences, Graduate School of Health Sciences, Gunma University, Japan

Objective: Mongolian National University of Medical Sciences (MNUMS), only one national medical university, and Gunma university (GU) have been continuing histopathological and cytopathological education and research based on the inter-university agreements from 2012. An overview of the support, results and future prospects is reported.

Background: Mongolian population is about 2.7 million. About half people live in the capital area and other half are nomads living in distant rural areas and this is one of the reasons for medical disparities as a major health problem.

Cancer incidence and mortality rate is increasing about 1.5 times in recent 20 years and about 80% is discover at progressed stage and construction of equal medical laboratory system is an urgent. Also the current curriculum for medical technologist students has been 3-year-course and histo- and cytopathology is not included and now under transition to 4-yeasr-course. So educational curriculum with histo- and cytopatology and with professional teaching staffs for medical technologists (MT) is necessary.

Summary and Results: In 2012, GU staffs visited MNUMS and pathological facilities and discussed on educational and pathological practice. From 2012 to 2015, two teaching staffs from MNUM visited GU and trained for educational skills on histo- and cytopathology and prepared teaching textbooks for MNUMS students of hospitals. In 2013, GU staffs visited MNUMS and lectured on histo- and cytopathology for the MT students for the first time at the school and gave some lectures for histopathology staffs around MNUMS. From 2015, a cooperative and comparative study on 'consciousness and attitude on cervical screening and HPV vaccination' is being conducted with teachers who have studied at GU.

Discussion: Delay of pathology, cytology field of developing countries is still outstanding and 'cancer screening' system, which have contributed significantly to the decrease in cancer mortality in Japan is not well organized yet and cancer is mortality is not reduced due to shortage of medical human resources and variation in medical laboratory system. To support sustainable hisotpthological and cytopathlogical activities local in Mongolia, it is necessary to foster professional human resources capable of providing education in the local. Also it is important to precise analysis of local health situation and was analyzed in detail and to understand and respect the needs and autonomy of the local need and independency.

Disclosure of Interest: None declared.

CT-5

Non-Hematolymphoid Metastatic Neoplasms to the Breast

Mei-ling Wu, Jen-sheng Ko, Li-sun Shih, Mei-hua Tsou

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Objectives: The breast lesion metastasis form extra-mammary malignancy is rare with the incidence of 0.4–1.3% and most of them are hematolymphoid neoplasm. To evaluate the cytological features of metastatic extra-mammary tumors could provide the accurate diagnosis and prevent unnecessary surgery.

Materials and Methods: Ten cases of non-hematolymphoid metastatic neoplasms to the breast were identified in a series of 18211 cytological specimens of the breast at Koo Foundation Sun Yat-Sen Cancer Center from 2006 to 2015. These specimen types included core biopsy imprint smears (4 cases), fine needle aspiration with cell block (2 cases), and the fine needle aspiration only (4 cases). All of these 10 cases were confirmed by ancillary studies performed on histology specimens or FNA materials of the breast lesions, including immunostains and the testing of Epstein-Barr virus.

Results: The ten patients comprised eight women and two men, with a mean age of 49 years (range of 33 to 67 years). The primary sites were varied including the lung (5 cases), prostate (1 case), thymus (1 case), ovary (1 case), pancreas (1 case) and nasopharynx (1 case). Small cell lung cancer and thymic carcinoma were the two cases which present as breast lesions with unknown primary malignancy initially. The cytological patterns and immunostain results are not only different from the primary breast cancer but also similar to the primary site of the previous malignant lesions and raised the suspicion of extra-mammary solid tumor involving the breast lesions.

Conclusion: Metastasis to the breast from an extra-mammary neoplasm usually indicates disseminated metastatic disease and a poor prognosis. The presence of unusual cytomorphological patterns on breast specimens should alert the cytopathologists the suspicion of a metastatic breast neoplasm. To avoid misdiagnose the extra-mammary malignancy metastasis to the breast, compare the morphology with primary malignancy is highly recommended. A detailed history of the patients, clinical correlation, and ancillary studies such as immunohistochemical or immunocytological profiles are useful to establish an accurate diagnosis and provide appropriate managements for these patients.

Disclosure of Interest: None declared.

CT-6

Cytology Interpretations of Atypical Endometrial Cells (ATEC) of Descriptive Reporting Format for Endometrial Cytopathology

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Background: Endometrial cytology is being most widely use in Japan as screening procedure. In addition, in the JSCC atlas and guidelines for cytopathological diagnosis, descriptive reporting format for endometrial cytological diagnosis is placed. Cytological results were classified as 'negative for malignancy', 'atypical endometrial cells' (ATEC), 'endometrial hyperplasia', 'atypical endometrial hyperplasia' or 'malignant tumor'. ATEC was subclassified as 'ATEC, of undetermined significance' (ATEC-US) and 'ATEC, cannot exclude atypical endometrial hyperplasia or more' (ATEC-A).

Cytological Findings of ATEC-US: Cell clumps with irregular protrusions composed of metaplastic cells. Most cases were histologically diagnosed as benign endometrium with abnormal hormonal influence by endometrial tissue sample. Usually, when many cell clumps composed of metaplastic cells are observed, definite evaluation is difficult.

Cytological Findings of ATEC-A: Cell clumps with irregular protrusions or dilated and branched patterns exist. Because of the inflammatory or the remarkable hemorrhagic background, precise cellular evaluation is difficult. Most cases were histologically diagnosed as atypical endometrial hyperplasia or endometrioid adenocarcinoma.

Conclusion: The descriptive reporting format for endometrial cytological diagnosis is the current diagnostic standards for endometrial cytopathology in Japan. The category of ATEC is useful adjunct in the diagnosis of suspicious endometrial lesions.

Disclosure of Interest: None declared.

CT-7

Birth of a New Cytopathology Service at Sidra – Doha, Qatar

Nikolaos Chantziantoniou

Sidra Medical and Research Center, Qatar

The Sidra Medical and Research Center (SMRC) is a new women's and children's hospital in Doha, Qatar, a small but significant sea-faring nation. SMRC vision: *Be a beacon of learning, discovery and exceptional care, ranked among the top academic medical centers in the world*; mission: *Provide patients with world class healthcare services in an innovative and ultramodern facility specially de-*

signed to promote healing. Address the growing need for more comprehensive patient focused medical services for women and children in Qatar and throughout the region. SMRC is characterized by a futuristic design, architecture, and enterprise-wide electronic network. Cytopathology is charged to serve tailored, culturally-sensitive diagnostic services: cervical (Pap) testing, fine needle aspiration biopsy, and body fluid analyses for opportunistic or neoplastic disease.

To accommodate the projected caseloads, the service is structured comprehensively upon Cellient, T5000 ThinPrep liquid based technology with computerized image based diagnostics. Throughputs also facilitate introductory cervical cancer screening, digital image mining, and HPV considerations.

Given the demographics and healthcare priorities characteristic of this region, SMRC actively partners with major institutions to establish foundational initiatives to mature the overall cytopathology profession, ensuring commensurate human capital to manage services. Initiatives include theoretical and practical training programs to produce candidate qualifications, and professional certification mechanisms to recognize knowledge and register credentials. The inaugural International Academy of Cytology certification examinations are scheduled at SMRC in early 2017. Dedicated symposia in 2014 celebrated 100 years of cytopathology development; future meetings will showcase current developments raising awareness of diagnostic scope of service data for all stakeholders.

Professional licensure is mandated though Qatari ministerial authorities. Practicing cytologists renew privileges periodically declaring required educational credits. Conventional glass slide educational and proficiency testing schemes are standard. Given the SMRC all-digital infrastructure, fully digital web-based platforms are favored testing cytologists' application of diagnostic templates through categorized cell images with educational support. We evaluated an innovative digital-image educational/PT module (BestCyte) developed by CellSolutions, LLC, facilitating immediate diagnostic feedback.

The presentation emphasizes personal experience in design and implementation of tailored cytopathology diagnostic platforms in the Gulf region. Consideration of the local disease incidence, perceptions, and cultural attributes is crucial.

Disclosure of Interest: No relationship. We evaluated a new innovative all-digital web-based educational/proficiency testing platform developed by CellSolutions for gynecological cytopathology. They have offered to support travel and hotel costs. This shall be mentioned in my presentation.

CT-8

The Analysis of Anorectal Washing Cytology in 46 Cases of Crohn's Disease

Satoko Ogata[1], *Masami Nambu*[1], *Shizuka Yamada*[1], *Ryo Hayashida*[1], *Hiroshi Tanabe*[1], *Keisuke Ikeda*[1], *Kitaro Futami*[2], *Seiji Haraoka*[1], *Akinori Iwashita*[1]

[1]Department of Pathology, Fukuoka University Chikushi Hospital, [2]Department of Surgery, Fukuoka University Chikushi Hospital, Japan

Objectives: Recently, the colitis associated cancer (CAC) has been increasing according to an increase of inflammatory bowel disease (IBD) that are Crohn's disease (CD), ulcerative colitis (UC) and so on. CD mainly attacks on young population and repeats remission and recurrence. There is recognized increased risk for colorectal cancer in patients with IBD, particularly in long-standing and extensive UC. There also appears to be an increased rate of intestinal cancer in CD, including both colon and small bowel sites. Therefore it is important to keep a periodical check of colon for an early detection of cancer. In our hospital, a washing cytology is performed as one of the periodical check. This time, we analyzed 46 cases of the anorectal washing cytology of the CD patients.

Materials and Methods: 46 cases of anorectal washing cytology of the CD patients performed in our hospital were analysed. Histological diagnosis was made in all the cases. The Papanicolaou class I to III was taken for benign and more than IIIb was taken for malignancy. The results of the washing cytology were compared with that of histology.

Result: In the 46 cases, 9 were malignant histologically, and 6 of them were Class IIIb to V cytologically, another one was Class II and the other two were Class III. The other hand, in the 37 cases histologically diagnosed non-carcinoma, there was no case of false-positive (more than Clas IIIb) by cytology. According to this, sensitivity is 66.7%, and specificity is 100%. About histological type of the 9 malignant cases, 4 cases were well to moderately differentiated adenocarcinoma, 4 cases were mucinous adenocarcinoma and another case was small cell carcinoma.

Conclusion: In Europe and America, the arising site of CAC in CD is frequent in right colon, the other hand, in Japan, about 60% cases arise in anal canal and rectum. Earlier detection of colorectal cancer based on colonoscopy screening for surveillance may be achieved but it has not translated into an effective approach because severe inflammation preventing from clear endoscopic image. Then the anorectal washing cytology is performed in our institution as an additional surveillance approach. About the anorectal washing cytology, it's easy for patients and possible to screen for a wide range of anorectal area. In the anorectal washing cytology, we need to make a careful observation on cellular atypia even if there are only a few epithelial cells, because many cases of CAC in CD are well-differentiated or mucinous adenocarcinoma with weak cellular atypia. If we note this point, the anorectal washing cytology might be useful for a surveillance to early detect CAC in CD patient.

Disclosure of Interest: None declared.

Workshops

WS01

Electronic Image Enhancement for Better Micrographs

*Daniel F. Iyama-Kurtycz**

University of Wisconsin School of Medicine and Public Health, United States

Summary: In a seminar format, The session instructs individuals on the theory and practice of electronic image manipulation using desktop/laptop computation. The most popular editing tool Adobe Photoshop®, will be employed, but other packages with similar capabilities will be mentioned. Characteristics of quality electronic photographs will be discussed. The session will show how to color balance, brighten and sharpen images. There will also show examples of what should not be acceptable for scientific images. Since all presentations are now given by means of computers and electronic projection systems there is a significant need to teach people how to optimize scanned or captured digitized images for public display.

After the Workshop:

1. Participants should be able to understand basic color theory and color correct their own images.
2. Participants should understand basic photographic histograms that define images in terms of highlights, mid-tones and shadows.
3. Further participants should be able to manipulate the histograms in order to improve their images.
4. Participants should be able to apply the concepts of sharpening to improve the clarity of their images.
5. Participants should understand the basic desktop tools of software such as Adobe Photoshop and use this knowledge to explore image improvement.

WS02

Glandular Lesions in Liquid-Based Pap Tests

*Rana S. Hoda**

New York Presbyterian Hospital, Weill Cornell Medical College, United States

Summary: This workshop will enable confident diagnosis of various reactive, benign and neoplastic glandular lesions on Pap Tests. The workshop discussion will evolve around 10 wide-ranging cases. Emphasis will be placed on morphological appearances of glandular lesions as seen on liquid-based preparations. Comparative analysis of various lesions on conventional smears, common artifacts, diagnostic dilemmas and potential pitfalls will be highlighted. The role of HPV-testing in endocervical lesions will be outlined. Current diagnostic and management guidelines will be reviewed. The importance of histological and clinical correlation will be emphasized. Related quality assurance issues will be addressed. An interactive approach with the audience, and brief question-answer and discussion periods will ensure audience participation. Practicing pathologists, pathologists-in-training and cytotechnologists will benefit from this course.

Upon completion of the course, the participants will be able to: 1) diagnose various glandular lesions on liquid-based Pap Tests; 2) recognize differences in appearance of these lesions on conventional and liquid-based preparations; 3) utilize ancillary tools, including immunocytochemistry and HPV-testing; 4) realize limitations of cytological diagnoses; and 5) understand current management guidelines.

WS05

Diagnostic Pitfalls in the Work Up of Efusions: The Real, the Pretender and the Mimicker

*Claire W. Michael**

Case Western Reserve University/University Hospitals Case Medical Center, United States

Summary: Despite the tremendous progress in cytology techniques and immunostains, effusion cytology remains a significant source of diagnostic difficulty and constitute a considerable portion of outside consultations. This presentation will establish the basics of effusion cytology diagnosis and demystify common and uncommon diagnostic pitfalls.

In this presentation, selected cases will be utilized to illustrate examples of the common diagnostic dilemmas encountered in effusion cytology, namely reactive mesothelium, adenocarcinoma and mesothelioma. The characteristic morphological features that assist the reader in distinguishing these entities will be discussed along with the best immunostaining (IMC) panels utilized to establish the correct diagnosis. Additional cases will illustrate uncommon malignancies that may mascarade as adenocarcinoma or mesothelioma such as squamous and urothelial carcinomas in effusions or malignancies that could be overlooked such as small cell carcinoma. Benign entities that may mascarade as carcinoma such as atypical histiocytes or endometriosis will also be discussed. The conference will conclude by a short review of best practices in the application of IMC in the work up of effusions.

WS06

Cytopathology of Infectious Diseases

Walid E. Khalbuss[1]*, *Pam Michelow*[2]

[1]Department of Pathology, University of Pittsburgh, United States; [2]University of the Witwatersrand and National Health Laboratory Service, South Africa

Summary: Micro-organisms are regularly encountered in cytology specimens. The identification of organisms by cytomorphology may be challenging. The incidence of infectious disease is increasing worldwide. This is due to several reasons including global travel, and immunosuppressive e.g. organ transplant, HIV/AIDS etc. It is necessary for the practicing cytopathologist and cytotechnologist, in both developed and low-resource nations, to acquaint themselves with the cytomorphology of a variety of infectious diseases, and the ancillary investigations required to confirm the diagnosis, in order to ensure the patient receives timely and appropriate antimicrobial management.

This workshop will comprise lecture, glass slides and virtual microscopy, and will focus on the identification of organisms, the differential diagnosis thereof and ancillary investigations to confirm the diagnosis.

Objectives:

- Describe the cytomorphology of selected common and uncommon infections, and their differential diagnosis.
- Discuss ancillary testing investigations to confirm the diagnosis.
- Review some contaminants and mimics of infectious organisms.

WS07

What a Cytopathologist Should Know on Next Generation Sequencing

Giancarlo Troncone[1]*, *Gilda da Cunha Santos*[2]

[1]Department of Public Health, Pathological Section, University of Naples Federico II, Italy; [2]University Health Network, University of Tronto, Canada

Summary: Molecular cytopathology has gene sequencing as its core technology. Until recently, cytological samples were only tested by sequential single-gene mutational tests. Today, with the better understanding of the molecular events involved in malignancy and of the mechanisms of pharmacotherapy, larger gene panels are more informative than a single biomarker. Next generation sequencing (NGS), matched with multiplex capture of targeted gene regions and analysed by sophisticated bioinformatics tools, enables the simultaneous detection of multiple mutations in multiple genes. By the development of miniaturized technology and of benchtop sequencers it is not unlikely that NGS will soon be adopted for routine molecular diagnostics, including cytological samples. This multinational workshop addresses (1) the most relevant methodological and technical aspects of the NGS analysis workflow and the diverse platforms available; (2) the issues related to daily practice implementation, namely, the cytological sample requirement and the validation procedures; and (3) the opportunities that NGS offers in different fields of cytopathology, to increase mutation detection sensitivity in paucicellular smears and to extend the analysis to a larger number of gene regions. Cytopathologists involvement and coordination in this rapidly evolving field is crucial for the effective implementation of NGS in the present and future cytological practice.

WS08

Lessons from the NHSCSP Audit of Invasive Cervical Cancer

John H.F. Smith, Nick Dudding*

East Pennine Cytology Training Centre, United Kingdom

Summary: Following a power point presentation describing the morphological lessons learned from central review of contentious SurePath and ThinPrep LBC cervical cytology specimens in the NHSCSP Audit of Cervical Cancer, participants will have the opportunity to give their anonymised opinion on a series of projected images of samples obtained prior to the diagnosis of invasive cervical cancer using an interactive electronic voting system (TurningPoint).

East Pennine Cytology Training Centre will provide the interactive voting technology (TurningPoint).

This workshop will be of interest and relevance to all cytotechnologists and cytopathologists reporting LBC cervical cytology specimens.

WS09

Cervical Cytology – Challenging Squamous and Glandular Lesions

Ritu Nayar[1]*, *David C. Wilbur*[2,3], *Donna K. Russell*[4]

[1]Northwestern University, Feinberg School of Medicine, [2]Harvard Medical School, [3]Clinical Imaging, Massachusetts General Hospital, [4]Cytopathology Residency/Fellowship Program, University of Rochester Medical Center, United States

The glass slides will include examples of challenging cases of benign, reactive and neoplastic squamous and glandular lesions and highlight pitfalls and differential diagnosis. All preparation types-conventional and liquid based preparations (ThinPrep and SurePath) as well as relevant histology will be included. Short power point presentations will be interspersed with the individual microscopic review and the three workshop faculty will be available for individual participant questions and discussion during the entire period.

Glass slide packets will contain liquid based and conventional preparations of both squamous and glandular lesions of the endo-

cervix. Endometrial and other malignancies will also be included. All packets will contain clinical information including patient follow-up. Selected cases will include cell block preparations and ancillary testing.

WS10

Cervicovaginal Cytology: Look-Alikes

Luigi Di Bonito[1]*, *Giovanni Negri*[2]

[1]Anatomic Pathology, Histology and Cytopathology, Eminent Scientist in University of Trieste, [2]Department of Pathology, Central Hospital Bolzano, Italy

The workshop will focus on cervicovaginal cytology including diagnostic criteria and look-alikes on conventional smears and liquid-based cytology. The session will include a brief presentation (about 30') with powerpoint slides and a microscopy session with representative glass slides.

The session will start with a short PowerPoint presentation followed by distribution of glass slides to examine under individual microscopes.

WS11

Molecular Diagnostics in Cytopathology Specimens

Sinchita Roy Chowdhuri[1]*, *Sharon B. Sams*[2]

[1]Department of Pathology, The University Of Texas M.D. Anderson Cancer Center, [2]Department of Pathology, University of Colorado, United States

Summary: This presentation would focus on molecular testing of cytopathology specimens. Discussion of various specimen types and preparations along with the key features of cytopathology specimens which are relevant for selection of material for molecular studies will be undertaken. In addition, the basics of molecular tests, the underlying technology, and the challenges and limitations of molecular testing will be discussed. Power-point didactic material will be presented to explain the key concepts, together with a discussion of how to select the best material for analysis and understanding the role of the cytopathologist in sending material for appropriate testing.

Increasingly, molecular testing is being requested in cytopathology material. Because of the variety in specimen types and preparations, it is important to understand the key features that define the best specimen for testing, and how to identify potential limiting factors within specimens which could confound molecular results. Recognizing factors that affect molecular testing will allow for selection of appropriate material for meaningful interpretable results that impact patient clinical management.

Learning Objectives:

- Understand the key specimen features which define best tissues for testing
- Understand the key features of molecular assays which interface with the specimen features
- Understand the role of the cytopathologist in forming the bridge between the requesting clinician and the molecular lab to facilitate the testing of optimal material

Core knowledge: 50%; Cutting edge knowledge: 50%.

WS12

Descriptive Reporting Format for Endometrial Cytology, Clinical Usage

Kenji Yanoh[1]*, *Yasuo Hirai*[2], *Yoshiaki Norimatsu*[3], *Tomohiko Yamaguchi*[4], *Azusa Hanada*[1]

[1]Departments of Obstetrics and Gynecology, JA Suzuka General Hospital, [2]Department of Obstetrics and Gynecology, Tokyo Women's Medical University, [3]Department of Medical Technology, Gaculty of Health Sciences, Ehime Prefectural University of Health Sciences, [4]Department of Pathology, Kurume University School of Medicine, Japan

In 2015, the new descriptive reporting format for endometrial cytology was published in the JSCC atlas and guidelines for cytopathological diagnosis (Vol.1). In this reporting format, cytological result were classified as 'negative for malignancy', 'atypical endometrial cells (ATEC)', 'endometrial hyperplasia', 'atypical endometrial hyperplasia', and 'malignant tumor'. ATEC was subclassified as 'ATEC, of undetermined significance' (ATEC-US) and 'ATEC, cannot exclude atypical endometrial hyperplasia or more' (ATEC-A). While a more detailed cytological diagnosis can be selected for cases of 'negative for malignancy', 'endometrial hyperplasia', 'atypical endometrial hyperplasia', or 'malignant tumor' with endometrial cytology, for cases evaluated as ATEC, either an ATEC-US or ATEC-A has to be selected without exception. In this reporting format, criterion for specimen adequacy was newly set. Moreover, according to diagnostic categories, clinical managements are set. These new designs enabled standardization of endometrial cytology in Japan.

WS13

Thyroid Fine-Needle Aspiration (FNA)

*Massimo Bongiovanni**

Institute of Pathology, University Hospital, Switzerland)

This work shop presents all aspects of thyroid FNA and covers all diagnostic categories according to the Bethesda Systems.

Molecular data, when available, are integrated into the diagnosis and help participants to formulate a morphological and molecular diagnosis based on malignancy risk stratification.

All cytological challenging diagnosis are supported by histological specimens.

WS14

Thyroid Cytopathology with Histopathologic Correlations: A Practical Approach

Syed Z. Ali, Justin A. Bishop*

Department of Pathology, The Johns Hopkins Hospital, United States

The pivotal role of fine needle aspiration (FNA) in the management of patients with thyroid nodule will be highlighted. Following a synergistic approach, the lecture/workshop will elaborate on diagnostic issues of practical importance and will describe the potential pitfalls leading to erroneous interpretation on FNA and histopathology. Cytomorphologic characteristics of common thyroid nodules will be discussed with reference to the Bethesda System for Reporting Thyroid Cytopathoogy (TBSRTC) and adequate histopathologic correlations will be presented. Clinical implications of the various cytopathologic interpretations will be discussed as well as the management guidelines for each diagnostic category. The crucial role of a multidisciplinary approach will be emphasized throughout the presentation. The workshop will be done using didactic lectures and unknown case presentation.

WS15

Soft Tissue and Bone FNA

*Henryk A. Domanski**

Division of Laboratory Medicine, Department of Pathology, Lund University, Sweden

Summary:

1. Approach to FNA of musculoskeletal neoplasm.
2. Brief review of cytological features of most common entities.
3. Application of ancillary tests in the FNA of soft tissue and bone neoplasm.

A major focus of this workshop will be on cytological evaluation and presentation of diagnostic criteria of most common entities of soft tissue and bone neoplasm. Important areas such as the multidisciplinary management of soft tissue and bone neoplasm, FNA vs. Core needle biopsy, and ancillary techniques will be covered. The goal of this workshop is to learn indications, advantages, pitfalls and limitations of FNA in the examination of musculoskeletal lesions and to improve the participant's ability to recognize common musculoskeletal neoplasm in FNA smears.

WS16

Cell Block and Beyond: High Yield Goal – How To?

*Vinod B. Shidham**

Department of Pathology, Wayne State University School of Medicine, Karmanos Cancer Center and Detroit Medical Center, United States

Summary: The role of cell blocks in cytopathology is increasing with advances and refined methodologies for evaluation of a few diagnostic cells in the specimens by molecular techniques and immunocytochemistry (IHC). However, routine random approaches for preparing cell blocks have been frustrating with higher potential for suboptimal results.

This topic is of extreme interest for all pathologists in general and cytopathologist/cytotechnologists in particular with relatively fragmented and confusing information in the literature. Based on direct feedback at many presentations, on ASC listserv, and as editor, many members are confronting the difficulty in this area on ongoing basis.

The current advances in cancer therapy with increasingly expanding range of personalized medicine have enhanced the demand for more diagnostic/therapeutic/prognostic tests on scant specimens such as cytopathology material for molecular pathology and immunohistochemistry. This is heavily dependent on good cell blocks. It is not uncommon that the only material available would be the initial cytopathology material and if the cell block generated is not of good quality (which with conventional approach is commonly suboptimal), the critical tests are not possible. Thus having good cell block in as many cases as possible would decrease/eliminate the need for additional preventable invasive procedures/interventions.

This 2 hour workshop will address issues leading to the frustrations and discuss alternatives to overcome many of these problems including methodical evaluation of immunocytochemistry results on cell block sections especially for the specimens with scant isolated diagnostic cells by Shidham's SCIP approach (1).

Brief Outline of Presentation:

a. General background.
b. Review of current methods and limitations.
c. Recent advances to overcome some challenges.
d. Brief comments related to ancillary tests such as IHC and molecular tests.
e. Question-Answer

Reference

1 Shidham VB, Atkinson BF: Immunocytochemistry of effusion fluids: introduction to the SCIP approach, in: Shidham VB, Atkinson BF (eds). Editors 'Cytopathologic Diagnosis of Serous Fluids' First edition, Elsevier (W.B. Saunders Company) 2007, pp 55–78.

WS17

Significance and Quality Control of Endometrial Cytology

Yoshiaki Norimatsu[1]*, *Kenji Yanoh*[2], *Yasuo Hirai*[3], *Tomohiko Yamaguchi*[4], *Hirofumi Sakamoto*[5]

[1]Department of Medical Technology, Faculty of Health Sciences, Ehime Prefectural University of Health Sciences, [2]Obstetrics and Gynecology, JA Suzuka General Hospital, [3]Department of Obstetrics and Gynecology, Tokyo Women's Medical University, [4]Department of Pathology, Kurume University Hospital, [5]The Tohkai Cytopathology Institute, Japan

Summary: Recently the Osaki Study Group (OSG) proposed new cytological diagnostic criteria that can be used with the standardised and automated sample processing methodology of BD SurePath-LBC system. This new cytological diagnostic criteria has been called: 'diagnostic flowchart of the OSG method', and is a simple diagnostic method consisting of three steps. The first step evaluates whether the morphology of the cell clumps is regular or irregular and whether nuclear overlapping amounts to more or less than three layers. Next, a cytomorphological evaluation of endometrial samples considering various physiological or physiopathological situations of the endometrial mucosa is performed and in the third step the final diagnosis is finally made. The OSG method terminology for final diagnosis consists of six categories: (1) normal endometrium, (2) Endometrial glandular and stromal breakdown, (3) atypical endometrial cells, cannot exclude atypical endometrial hyperplasia or more (ATEC-A), (4) adenocarcinoma including atypical endometrial hyperplasia or malignant tumour, (5) endometrial hyperplasia without atypia (EH), (6) Atypical endometrial cells of undetermined significance (ATEC-US).

WS18

Dysplasia in Biliary Tract Brushings of PSC-Patients

Leena Krogerus[1]*, *Johanna Arola*[2]

[1]HUSLAB, Finland; [2]Helsinki University Hospital, Sweden

Summary: Following a Power Point presentation describing the morphological lessons learned from comparing cytological morphology from biliary tract brushings, DNA-cytometry and subsecuent histological specimens of patients with primary sclerosing cholangitis, participants will have the opportunity to give their anonymised opinion on a series of projected images of brush cytology samples obtained prior to the diagnosis of dysplasia or invasive cancer of the biliary tract using an interactive electronic voting system (provided by the Finnish Medical Association Duodecim).

This workshop will be of interest and relevance to all cytopathologists reporting LBC brush cytology specimens from the biliary tract.

WS19

Look-A-Likes in LBC

Minaxi Sharad Desai[1,2,3]*

[1]Central Manchester University Hospitals, [2]Institute of Cancer Sciences, University of Manchester, [3]School of Healthcare Sciences, Manchester Metropolitan University, United Kingdom

Summary: In this session I intend to provide Thinprep and Surepath LBC material which illustrate examples of the squamous and glandular lesions showing similar morphology in a diverse group of lesions which may cause difficulty in making the final diagnosis. The session will start with a short PowerPoint presentation followed by distribution of the slides to examine under individual microscopes.

WS20

Urinary Cytopathology: An Update on Terminology, Morphology and Clinical Management

Christopher Vanden Bussche[1]*, *Ashish Chandra*[2]

[1]Department of Pathology, The Johns Hopkins University School of Medicine, United States; [2]Guy's and St. Thomas Hospital NHS Foundation Trust, United Kingdom

Summary: Interpretation of urinary cytology specimens from the upper tract (renal pelvis and ureters) are fraught with errors due to lack of familiarity with the range of cytomorphological appearances and sometimes also to the lack of clinico-radiological information available at the time of reporting these specimens. The tutorial will cover the range of appearances in inflammatory lesions, calculous disease, low and high grade urothelial neoplasia, correlation with accompanying biopsies and the approach to reporting these specimens.

The tutorial would be suitable for trainee and consultant pathologists reporting urinary tract cytology specimens and also for cytotechnologists involved in the screening of urine cytology specimens. Those with a specialist interest in urological cytohistopathology would be welcome to share their experience in his interactive session.

WS21

Lymph Node Fine Needle Cytology

Pio Zeppa[1]*, *Gilda da Cunha Santos*[2]

[1]Department of Pathology, University of Salerno, Italy; [2]Department of Laboratory Medicine and Pathobiology, University of Toronto, University Health Network, Canada

Summary: This Workshop is addressed to cytopathologists who desire to upgrade their professional knowledge on basic lymph node cytopathology and its main issues. The workshop will

start with a very brief introductive lecture; then a large and varied selection of paradigmatic cases will be provided to the participants using glass slides combined with relevant clinical data. Principal and infrequent pathological entities will be shown, along with their cytological presentations, the diagnostic algorithms, the usage of ancillary techniques and the cyto-histological correlations. Attendees will be assisted on request with 'hands on' participation.

WS22

A Practical Pattern Based Diagnostic Approach to Salivary Gland Fine Needle Aspiration Cytology

Longwen Chen, Matthew A. Zarka*

Division of Anatomic Pathology, Mayo Clinic Arizona, United States

Summary: Fine needle aspiration (FNA) biopsy is widely accepted as part of the initial evaluation of salivary gland lesions. It is cost-effective, fast, and safe. The results of FNA biopsy of the salivary gland lesions can significantly affect patient management, for instance, by distinguishing salivary gland neoplasia from inflammatory lesions.

However, FNA biopsy of salivary gland lesions can be very difficult, mainly due to overlapping cytological features among low grade salivary gland neoplasms. The heterogeneity of many salivary gland tumors in many cases limits the accuracy of subtyping on small FNA biopsies. Thus, the practice of FNA cytology of salivary gland remains a significant challenge to many cytopathologists.

Novel point of this proposed work shop:

To address the challenges listed above, we propose a novel pattern based approach to the diagnosis of salivary gland lesions on FNA.

Major education objectives of the work shop:

A. Present a practical pattern based diagnostic approach to common salivary gland lesions encountered in daily cytopathology practice;
B. Review the current roles of salivary gland FNA biopsy in the clinical management of patients;
C. Identify the limitations of FNA biopsy in the diagnosis of salivary gland lesions;
D. Provide a current overview of ancillary tests (including molecular tests) in the cytological diagnosis of salivary gland neoplasms.

WS24

Pancreas Cytology Made Simple Using an Algorithmic Approach

Nirag C. Jhala[1]**, Darshana Jhala*[2]

[1]Temple University Hospital, [2]University of Pennsylvania, United States

Summary: Pancreas cytology remains one of the challenging samples for any cytology personnel. This microscopy workshop will demonstrate how an algorithmic approach can help assessments for pancreatic cytology samples.

At the end of the workshop participants will be able to observe and use morphologic approaches to diagnosis of pancreatic carcinoma and its varied morphologies and distinguish them from chronic pancreatitis. The workshop will also demonstrate how non-ductal pancreatic cancers such as pancreatic endocrine tumour can be differentiated from its common mimicker like solid pseudopapilary neoplasm of the pancreas. The workshop will demonstrate approach to various cystic lesions of the pancreas including mucinous cysts of the pancreas. The participants will also understand use and value of judicious use of ancillary testing in aiding diagnosis of various pancreatic lesions.

WS25

Directly Sampled Endometrial Cytology (Conventional and Liquid-Based Endometrial Cytology)

Yuko Sugiyama[1]**, Kyoko Komatsu*[2]

[1]Department of Cytopathology and Gynecology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, [2]Cancer Institute Hospital, Japanese Foundation for Cancer Research, Japan

Summary: This workshop will focus on the microscopic finding of directly sampled endometrial cytology with more than 30 cases of glass slides (Conventional and liquid-based endometrial cytology).

The content of this workshop as follows:

A. Device and procedure of directly sampled endometrial cytology (Conventional and liquid-based endometrial cytology).
B. Reporting format and diagnostic criteria of endometrial cytology.
C. Microscopic findings of endometrial cytology.
1. Benign lesions
   Proliferative, Secretory, Atrophic endometrium, etc.
2. Precursors
   Endometrial hyperplasia, Endometrial hyperplasia with atypia
3. Endometrial carcinomas
   Endometrioid carcinoma, Serous carcinoma, Clear cell carcinoma, etc.
4. Mesenchymal tumors
5. Mixed epithelial and mesenchymal tumors

D. Microscopic findings of conventional and liquid-based endometrial cytology.

WS26

Molecular Cytopathology – Pre-Analytical and Technical Requirements for Molecular Testing Using Cytological Samples

Gilda da Cunha Santos[1]*, *Giancarlo Troncone*[2], *Claudio Bellevicine*[2]

[1]University Health Network. University of Toronto, Canada;
[2]Department of Public Health, Pathological Section, University of Naples Federico II, Italy

Summary: This advanced multinational workshop will review the current guidelines for molecular testing for different sites with special focus on pre-analytical parameters and provide practical guidance to cytopathologists about the routine requirements for different DNA mutational tests. Emphasis will be made on the following topics: sampling methods, type and source (primary vs. metastatic) of cytological samples suitable for molecular analysis and the differences among them, types of fixatives, the use of stained slides and the effect of cell preservation on the results, minimal cellularity and tumor cell enrichment, adequate DNA quantity and quality and methods for their assessment, sample processing (cell blocks) and how to maximize the yield, technical overview of the currently used assays and the specifics and the validation procedures of the analysis focusing on next generation sequencing (NGS), rates of successful assays of published series and how it compares to histological samples, how to timely refer a specimen for central testing and integrating the molecular reports to routine cytological reports. A case discussion approach will be used to illustrate practical aspects of basic principles and application of different cytological preparations for a variety of molecular analyses. Application of cytological material to future studies and specimen biobaking will also be discussed.

The workshop is designed for residents, pathologists and cytotechnologists with an interest in molecular pathology and looking for an update in recent advances in molecular tests applied to cytological samples. Information presented will be of value to professionals who want to improve the quality of specimens being obtained/received in their laboratories for optimal morphological and molecular analysis.

WS27

NSCLC Subtyping and Predictive Molecular Marker Analyses

*Lukas Bubendorf**

Institute of Pathology, University Hospital Basel, Switzerland

Summary: Current clinical practice of NSCLC subtyping, and predictive marker analysis, and technical aspects of biomarker analysis in cytological specimens will be outlined in a short introductory lecture. The participants will then have the opportunity to view a collection of cases at the microscope including the whole spectrum of specimens that have been used for biomarker analysis. A special emphasis will be placed on adequate NSCLC subtyping and selection of cytological specimens for next-generation-sequencing, including estimating the percentage of tumour cells, methods for enrichment, and integrative interpretation of NGS results. The PROs and CONs of conventional cytological slides and cellblocks, respectively, for subtyping and predictive marker analysis will be demonstrated. After this workshop, the participants will have a good understanding on how cytological specimens can successfully be exploited for predictive marker analysis.

WS28

Breast FNAC with an Emphasis on Papillary Lesions

Torill Sauer

Department of Pathology, Akershus University Hospital, Norway

Summary: A short introductory lecture about FNAC of papillary lesions of the breast (15 minutes).

Microscopy workshop: 'ordinary' benign and malignant cases and many cases of papillary lesions, both benign and malignant for microscopy.

WS29

Borderline (Atypical) Squamous Cells as an Inevitable Feature of Cervical Cytology

*Amanda Herbert**

Guy's and St. Thomas' NHS Foundation Trust, United Kingdom

Summary: In the great majority of cases it should be possible to distinguish normal and reactive changes in cervical cytology from high-grade or low-grade squamous intraepithelial lesion (HSIL and LSIL) but it is inevitable in a screening test, when so much attention must be paid to not missing significant abnormalities while false positives must also be avoided, a borderline category is inevitable.

The terms atypical or borderline should be used for cases in which there is genuine doubt as to whether the changes are reactive, SIL or cancer; in practice, atypical squamous cells of undetermined significance (ASC-US) and its equivalent category in the UK terminology 'borderline changes in squamous cells, not otherwise specified (NOS)' tend to be the categories most frequently used. Most ASC and borderline changes 'border' on reactive changes, LSIL or HPV infection but an important minority borders on HSIL and cancer: ASC, cannot exclude HSIL (ASC-H) and borderline, cannot exclude high-grade dyskaryosis respectively.

High-risk HPV triage can help solve the dilemma of ASC and borderline reports, but cannot tell productive from persistent infection. High-risk HPV is common and may be found in samples with benign reactive changes; sensitivity of high-risk HPV is less than perfect and negativity does not exclude HSIL or cancer. Accurate cytology remains important even with high-risk HPV triage.

WS30

FNA in Thyroid

Mitsuyoshi Hirokawa[1]*, *Miyoko Higuchi*[2], *Ayana Suzuki*[2]

[1]Department of Pathology and Cytology, Kuma Hospital,
[2]Department of Laboratory, Kuma Hospital, Japan

Summary: Fifty kinds of thyroid diseases will be available. Each case contains both conventional and LBC smears, associated with HE-stained histologic sections. Additionally, some cases have immunocytochemically-stained smears. We will prepare a handout including a summary of the cytological findings and representative photographs for the attendants.

WS31

Whole Slide Imaging (WSI) and Cytology: Advantages and Drawbacks with Live Demonstration

Walid E. Khalbuss[1]*, *Daniel F. Iyama-Kurtycz*[2]

[1]Department of Pathology, University of Pittsburgh,
[2]University of Wisconsin School of Medicine and Public Health, United States

Summary: Whole slide imaging (WSI) has become an important modality in Digital Pathology and cytopathology. This technology allows entire glass slides to be digitized (scanned), producing an interactive digital image that can be examined in a manner simulating light microscopy. WSI are being used for education; telecytology, quality assurance (QA); archiving rare and interesting cytology cases, a well as, legal cases. The aim of this workshop is to review WSI technology, applications and limitations specific to cytopathology field. This workshop will have a live demonstration on these selected applications.

Objective:

- To introduce the participants to cytopathology informatics.
- To review digital imaging and its applications in the field of cytopathology.
- To discuss role of WSI in education; telecytology; archiving rare and interesting cases; QA; and diagnoses.
- To perform live demonstration of selected applications.

Video Microscopy Tutorials

VMT01

ThinPrep Cervical Cytology: Challenging Cases with Histologic Correlation and Clinical Management

*Christine Bergeron**

Department of Pathology, Laboratoire Cerba, France

Summary: It will be an interactive VMT with the presentation of 6 challenging cases on cervical squamous and glandular diagnoses. The differential diagnoses will be discussed. The management will be proposed and the final histological diagnosis will be presented.

VMT02

Cytology of EUS-FNA of the Pancreas

*Barbara A. Centeno**

Moffitt Cancer Center, United States

Summary: EUS-guided FNA is the preferred method of sampling pancreatic masses. Clinical and imaging findings are important to establishing the algorithmic approach. Challenges include the distinction of benign/reactive processes from adenocarcinoma, the recognition of nonductal neoplasms, and the classification of cystic lesions. This case based slide review will focus on practical challenges in the diagnosis of solid and cystic masses of the pancreas. Pitfalls, the application of ancillary studies, and terminology issues will also be discussed.

VMT03

FNAB of Lymph Nodes: The Diagnosis of Lymphoma, the Role of Core Biopsies and Ancillary Tests

*Andrew S. Field**

Notre Dame University Medical School and St. Vincents Hospital, Australia

Summary: Cases will be presented by powerpoint and videomicroscopy to demonstrate the diagnosis of lymphomas by FNAB of lymph nodes, correlating with core biopsy findings and emphasizing the role of ancillary testing which varies from lymphoma to lymphoma. The problem areas of small cell lymphoma, Grade 1 and 2 Follicular lymphoma and T cell lymphomas will be illustrated, and the importance of the role of flow cytometry, cytogenetics and core biopsy will be discussed. The importance of an integrated multidisciplinary approach will be emphasized.

VMT04

Atypia in Urine Cytology: What Should and Shouldn't Be Reported as Atypical and Why?

*Eva M. Wojcik**

Loyola University, United States

Summary: The term 'atypia', although not well characterized, is widely used in cytopathology. Until the Paris System for Reporting Urinary Cytology, there has not been a standardized definition of 'atypia' in urine cytology. Since the category has been mostly used as a 'waste basket', the reported rate of atypia ranges from 1.9% to 23%. During this microscopic session we will review a number of cases with cytomorphologic findings associated with known and specific causes that potentially could be called 'atypical'. These include umbrella cells, seminal vesicle cells, pseudopapillary clusters in instrumented urinary tract specimens, reactive changes due to stones, characteristic changes for infectious processes, treatment effects and urinary diversion specimens. The participants will review cases showing these morphologic findings and understand, why once the morphologic changes in urine specimens can be attributed to specific etiologic factors, these specimens should no longer be classified as 'atypical'. In the second part of the session, we will present cases that fit the criteria for the 'Atypical Urothelial Cells – AUC' category as defined in the new Paris System. In addition, AUC cases will be compared to cases categorized as 'Suspicious for High Grade Urothelial Carcinoma' (SHGUC) and High grade Urothelial Carcinoma (HGUC). The criteria for all these categories will be highlighted, and outcome data will be discussed.

VMT05

Upper Urinary Tract Neoplasia: Cytomorphology and Interpretation in Clinical Context

*Ashish Chandra**

Guy's and St. Thomas' Hospitals NHS Foundation Trust, United Kingdom

Summary: Interpretation of urinary cytology specimens from the upper tract (renal pelvis and ureters) are fraught with errors due to lack of familiarity with the range of cytomorphological appearances and sometimes also to the lack of clinico-radiological information available at the time of reporting these specimens. The tutorial will cover the range of appearances in inflammatory lesions, calculous disease, low and high grade urothelial neoplasia, correlation with accompanying biopsies and the approach to reporting these specimens.

VMT06

A Walk Around the Block

*Donna K. Russell**

University of Rochester Medical Center, United States

Summary: Update your knowledge of body fluids and image-guided fine needle aspirations requiring immediate assessment and specimen triage. Recognize the importance of cell block preparation. Radiologic findings, clinical history and cytologic criteria will be presented from a variety of contemporary image-guided techniques. Case studies will be discussed, along with ancillary testing. Techniques include endoscopic ultrasound-guided FNA, endoscopic ultrasound guided FNA, Super Dimension bronchial FNA, Cat-scan image-guided FNA and US-guided fine needle aspiration. This workshop will allow participants to interact with each case presented. The cases will include clinical history, radiologic findings and cytologic criteria. The correct interpretation along with surgical pathology or additional ancillary testing will be provided for each case.

VMT07

The Problems of ASC-US, AGUS and Metaplasia in Gynecologic Cytopathology

Daniel F. Iyama-Kurtycz[1]*, *Darshana Jhala*[2]

[1]University of Wisconsin School of Medicine and Public Health, [2]Clinical Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine, United States

Summary: In cervical cytology, the 'gray zones' of Atypical Cells of Uncertain Significance (ASC-US), Atypical Squamous Cells of Uncertain significance (ASC-US), and definitions of benign squamous metaplasia continue to be a source of frustration for cytologists, clinicians and patients. It is well known that a strictly morphologic analysis cannot totally resolve the diseased and the non-diseased population when evaluating the cytology of the uterine cervix for neoplasia. Many of us who perform microscopic analysis of these samples do not have a firm understanding the nature of ASC-US, AGUS and the protective reaction that is cervical metaplasia. This tutorial will aim to provide structure to these atypical categories and explain the morphologic overlap with some high grade intraepithelial lesions. In so doing the workshop attempts to bring some clarity to the Pap test. Information from important authors of the past as well as more recent works will be reviewed.

VMT08

Diagnosing Lymphomas Using Multiparameter Approach

*Nancy P. Caraway**

The University of Texas MD Anderson Cancer Center, United States

Summary: Fine needle aspiration of lymphoproliferative disorders can be challenging. In this workshop selected cases of lymph node aspirates with an emphasis on commonly occurring lymphomas will be presented. The cytomorphologic features will be assessed and a differential diagnosis will be discussed. Ancillary studies including flow cytometric analysis and molecular studies will be integrated in the work-up as appropriate. Furthermore, potential pitfalls and limitations in the diagnosing lymphomas by FNA will also be discussed.

VMT09

Colposcopy and Cytopathology

Masatsugu Ueda[1]*, *Tetsuya Muroya*[2], *Toshitada Ogasawara*[3]

[1]Cytopathology and Gynecology, Osaka Center for Cancer and Cardiovascular Disease Prevention, [2]Department of Gynecology, Genkiplaza Medical Center for Health Care, [3]Department of Gynecology and Cytopathology, Akashi Ookubo Hospital, Japan

Summary: Colposcopy is a medical diagnostic procedure to examine an illuminated, magnified view of the cervix, vagina and vulva. Many premalignant and malignant lesions in these areas can be detected through the examination. Colposcopy provides an enlarged view of the areas, allowing the colposcopist to visually distinguish normal from abnormal appearing area and take directed biopsies for further pathological examination. Immediate colposcopy and biopsy would be recommended for women with abnormal cervical cytology of ASC-H, low grade SIL (LSIL), HSIL and atypical glandular cells (AGC). The main goal of colposcopy is to prevent cervical cancer by detecting precancerous lesions early and treating them. Low-grade CIN is often seen as thin, smooth acetowhite lesions with well-demarcated, but irregular, feathery or angular margins. High-grade CIN are associated with thick, dense, dull, opaque acetowhite areas with well-demarcated, regular margins. Vascular features, such as fine punctation and/or fine mosaics in acetowhite areas, may be associated with low-grade CIN. Coarse punctation and/or coarse mosaics in acetowhite areas tend to occur in high-grade lesions. Appearance of atypical blood vessels may indicate the first sign of invasion. In this program, typical colposcopic appearances and related cytological and histological findings will be presented based on the new 2011 colposcopy terminology.

VMT10

Directly Sampled Endometrial Cytology for Endometrial Cancer Screening

*Yuko Sugiyama**[1], *Kyoko Komatsu*[2], *Junzo Fujiyama*[2]

[1]Department of Cytopathology and Gynecology, Cancer Institute Hospital, [2]Department of Cytopathology, Cancer Institute Hospital, Japan

Summary: Currently, directly sampled endometrial cytology is the most common method of screening for endometrial cancer in Japan. This tutorial will focus on the detection of endometrial cancer by use of the directly sampled endometrial cytology.

The content of this video microscopy tutorial as follows:

A. Device and procedure of directly sampled endometrial cytology (Conventional and liquid-based endometrial cytology).
B. Reporting format and diagnostic criteria of endometrial cytology.
C. Detection rate between endometrial cytology and biopsy for diagnosis of endometrial cancers.
D. Microscopic findings of endometrial cytology.
1. Precursors
   Endometrial hyperplasia, Endometrial hyperplasia with atypia
2. Endometrial carcinomas
   Endometrioid carcinoma, Serous carcinoma, Clear cell carcinoma etc.
3. Mesenchymal tumors
4. Mixed epithelial and mesenchymal tumors

E. Microscopic findings of conventional and liquid-based endometrial cytology of endometrial cancer.

VMT11

Cervical Cytology in a Multidisciplinary Setting

Amanda Herbert[1]*, *Jaroslava Duskova*[2]

[1]Guy's and St. Thomas' NHS Foundation Trust, United Kingdom; [2]Charles University, Czech Republic

Summary: Cervical cytology is frequently carried out alongside high-risk HPV testing as a low-grade or ASC-US triage test or as a test of cure. In many countries vaccination will greatly reduce the prevalence of HSIL, LSIL and cervical cancer, which could compromise the accuracy of cytology and may lead to primary HPV testing being introduced. In view of the low specificity of HPV testing, cytology would still be needed as a triage test – and may be used as a primary co-test in some countries.

Whatever the nature of the primary and triage tests, colposcopists are faced with deciding which lesions need to be treated, which followed up and which returned to routine screening. Punch biopsies inform this decision but must be taken in the context of sampling, inter-observer variability and ancillary tests.

Women benefit from decisions made in a multidisciplinary setting in which the sensitivity and specificity of all modalities are

understood and can be discussed, reviewed and acted on rationally. No test is perfect but the combination of these tests can prevent the vast majority of cervical cancers by effective treatment of lesions most at risk of progression.

VMT12

Useful Molecular Cell Biological Techniques for Cytology

Kyoko Komatsu[1]*, *Yoko Nakanishi*[2], *Shinobu Masuda*[2]

[1]Cancer Institute Hospital, [2]Division of Oncologic Pathology, Department of Pathology and Microbiology, Nihon University School of Medicine, Japan

Summary: Molecular techniques for cytology materials: FISH, CISH, RT-PCR, Real-Time PCR, Gene Mutation assay, sequencing with useful cases.

Technical information: Study on minimum sample for PCR, RT-PCR, DNA and RNA Gene extraction with different LBC solution, result interpretation, and point to notes for gene analysis technique.

Immunocytochemical study of cytology samples: Sample preparation (Transcription, fixative, thickness of smear), effect of decolorization method.

VMT13

The Last Project – Polishing the Gold Standard

*Teresa M. Darragh**

Pathology and Obstetrics, Gynecology and Reproductive Sciences, University California, United States

Summary: The LAST Project, jointly sponsored by the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology, provides consensus recommendations for a unified histopathologic terminology for HPV-associated squamous lesions of the lower anogenital tract. Analogous to the Bethesda System for cervical cytology, the LAST Project is based on the principles that terminology must communicate clinically relevant information from the laboratory to the patient's health-care provider, be uniform and reasonably reproducible across different pathologists and laboratories and also be flexible enough to be adapted in a wide variety of settings and geographic locations. Lastly, terminology must reflect our most current understanding of HPV-associated neoplasia.

In this videomicroscopy tutorial, the basic tenets underlying the LAST Project will be reviewed and the recommendations for squamous intraepithelial lesions and the use of biomarkers to enhance our diagnostic reproducibility and accuracy will be illustrate using biopsy material from the lower anogenital tract.

Darragh TM, Colgan TJ, Cox JT, Heller DS, Henry MR, Nayar R, Palefsky JM, Stoler MH, Wilkinson EJ, Zaino RJ, Wilbur DC: The Lower Anogenital Squamous Terminology Standardization Project for HPV-associated Lesions: Background and Consensus Recommendations from the College of American Pathologists and the American Society for Colposcopy and Cervical Pathology.

References

J Low Genit Tract Dis 2012;16:205–242.
Arch Pathol Lab Med 2012;136:1266–1297.
Int J Gynecol Pathol 2013;32:76–115.

VMT15

Glandular Lesions in Cervicovaginal Cytology as Seen on Conventionals, ThinPrep and SurePath Preparations

*Dina R. Mody**

Cytology Laboratories, Houston Methodist Hospital and Bioreference Laboratory, United States

Summary: This one and a half hour microscopic tutorial will review examples of significant glandular lesions in cervicovaginal cytology using both conventional and liquid-based preparations (ThinPrep and SurePath). The lesions include adenocarcinoma in situ of the cervix, endocervical adenocarcinoma, endometrial and extrauterine carcinomas. The findings of these significant lesions will be contrasted with other benign mimics like tubal metaplasia, microglandular hyperplasia, directly sampled lower uterine segment endometrium and other artifacts and pitfalls. Criteria to separate high-grade squamous intraepithelial lesions from adenocarcinoma in situ will also be presented. Time permitting, selected consultations will be reviewed.

Target Audience: Pathologists, pathology residents, fellows and cytotechnologists and cytotechnology students.

Learning Objectives: At the conclusion of the course, participants should be able to: 1) list the diagnostic criteria for endocervical adenocarcinoma in situ and invasive adenocarcinoma, 2) describe the morphologic differences between a high-grade squamous intraepithelial lesion and endocervical adenocarcinoma in situ, and 3) identify the benign mimics of glandular neoplastic lesions in cervicovaginal cytology.

VMT16

Thyroid Fine Needle Aspiration Cytology and Liquid-Based Techniques

*Beatrix Cochand-Priollet**

Cochin Hospital University Paris Descartes, France

Summary: The objectives of this videocytology tutorial are:

1. To propose a complete overview of all the diagnostic categories on thyroid FNAs slides prepared with liquid-based techniques (Hologic and Becton Dickinson).
2. To analyze the morphological changes induced by these techniques and the more frequent subsequent pitfalls.
3. To illustrate some examples of immunocytochemistry applied on these slides.
4. To discuss with the participants about the diagnoses as well as about the terminologies.

VMT17

New Method to Restore Fading Papanicolaou Specimen Using an Optical Clearing Agentx

*Kiyotada Washiya**

Tokyo Central Pathology Laboratory Corporation, Japan

Summary: Cytological specimens are used only once for staining, and cannot be reused after preservation in paraffin blocks. If a technique is developed allowing restaining of precious specimens or those for education, which have been discolored due to long-term preservation, to restore their former states, it will be extremely useful for the reuse of specimens. We performed this study to determine whether discolored specimens can be restored to their former condition by applying an optical clearing agent.

Using the optical clearing agent (SCALEVIEW-A2), Papanicolaou (Pap) restaining, endurance tests of the optical clearing agent by repeated use, and immunostaining were performed.

After treatment with the optical clearing agent for 5–10 minutes, discolored cytological specimens could be stably restained just as the initial Pap specimens. And as a result of the immunostaining of specimens treated using this method, both the nucleus (Ki-67) and cell membrane (MOC31) were positively stained. The optical clearing agent did not deteriorate when used repeatedly ten times.

Discolored precious cytological specimens could be restored using the optical clearing agent. Specimens processed using this method could also be immunostained, and so this method is extremely useful.

VMT18

Pulmonary Cytology: Diagnostic Challenges and Optimal Use of Immunocytochemical Stains

*Hormoz Ehya**

Fox Chase Cancer Center, United States

Summary: The new targeted therapies require accurate classification of lung tumors. Improved survival in cancer patients has led to the increased occurrence of second and third primaries, necessitating the use of immunocytochemical stains to determine the primary source. Furthermore, the increasing need for molecular studies mandates prudent use of immunostains in order to conserve tissue. In this video microscopy tutorial the morphologic criteria to distinguish benign from malignant lesions and appropriate use of immunostains in the differential diagnosis of lung tumors are demonstrated.

Meet the Experts

ME01

Endocervical Glandular Lesions Showing Gastric Differentiation: Morphologic Spectrum and Concepts

*Yoshiki Mikami**

Department of Diagnostic Pathology, Kumamoto University Hospital, Japan

Summary: A variety of endocervical glandular lesions showing gastric morphology and phenotype, have been described in this decades, encompassing benign, borderline, and malignant conditions. Gastric-type adenocarcinoma (GAS), introduced in the recently revised WHO classification (4th ed., 2014) as a subtype of mucinous carcinoma, is a distinct entity with aggressive clinical behavior, compared with usual-type endocervical adenocarcinoma, and includes minimal deviation adenocarcinoma (MDA), also known as adenoma malignum, as its extremely well-differentiated variant. There are evidences indicating that GAS is closely related to lobular endocervical glandular hyperplasia (LEGH)/pyloric gland metaplasia (PGM), and importantly the GAS-LEGH/PGM sequence represents a non-HPV-related pathway of carcinogenesis. From the practical point of view, it should be kept in mind that GAS is a pitfall of HPV DNA-targeted strategy for early detection and vaccination for prevention. Therefore, cytotechnologists and pathologists appear to play a crucial role for detection of GAS, and thus should be familiar with its morphologic features. In addition, for the purpose of optimal management of GAS and LEGH, an intimate cross-talks between cytologists, pathologists, radiologists, and gynecologic oncologists are mandatory. This seminar provides updated information on current concepts and controversies on GAS, LEGH, and related lesions.

ME02

Intraoperative Cytology: An Old Technique for a New Era

*Steven G. Silverberg**

University of Maryland School of Medicine, United States

Summary: Intraoperative cytology (IOC) antedates most other cytopathologic techniques, having first been utilized in the 1920s. It was (and still is) used as a complement, or sometimes a replacement, for frozen section (FS) when an immediate diagnosis is requested in the Operating Room. Fresh specimens can be imprinted (touch preparation), smeared directly onto a slide, scraped with a sharp blade and smeared on a slide, or processed by other similar techniques. The resulting slides are either air-dried and stained with Giemsa or similar stains, or immediately alcohol-fixed and stained with H&E or Papanicolaou stain. The choice of fixation and staining techniques must be made in advance, and depends largely on the type of specimen (e.g., air-dried Giemsa for parathyroids, alcohol-fixed H&E for brain tumors). The techniques and their indications and contraindications will be discussed in detail. For most types of specimens, the accuracy of IOC is comparable to that of FS, and IOC has the additional advantage of being more rapid, especially when dealing with large or multiple specimens. IOC is also the technique of choice for extremely small biopsies (better preservation for permanent sections/special stains) and for necrotic and/or infected specimens. Examples of various specimens diagnosed intraoperatively will be presented.

ME03

Detection and Treatment of Pre-Invasive Diseases of the Cervix – With Special Reference to Colposcopy, Cytology and Laser Therapy

*Masatsugu Ueda**

Cytopathology and Gynecology, Osaka Center for Cancer and Cardiovascular Disease Prevention, Japan

Summary: Uterine cervical cancer is the second most common cancer in women worldwide, and is both a preventable and a curable disease especially if identified at an early stage. Cervical intraepithelial neoplasia (CIN) is the potentially premalignant transformation of squamous cells on the surface of the cervix. The major cause of CIN is chronic infection of the cervix with the sexually transmitted human papillomavirus (HPV). Some types of HPV can make women more likely to develop CIN and may lead to the development of cervical cancer. These are known as high-risk types, such as HPV types 16 and 18. CIN is usually discovered by a screening test, the Pap smear, reported using the Bethesda System. Immediate colposcopy and biopsy would be recommended for women with abnormal cervical cytology of ASC-H, LSIL, HSIL and AGC. Colposcopy is a medical diagnostic procedure to examine an illuminated, magnified view of the cervix. Colposcopy provides an enlarged view of the cervix, allowing the colposcopist to visually distinguish normal from abnormal appearing area and take directed biopsies for further pathological examination. The main goal of colposcopy is to prevent cervical cancer by detecting precancerous lesions early and treating them. Treatment for higher grade CIN involves removal or destruction of the neoplastic cervical cells by laser cautery, loop electrical excision procedure (LEEP), or cervical conization.

ME04

Pancreatic Neuroendocrine Neoplasms (PNEN): Pathology, Cytology and Genomics Update

*Robert Y. Osamura**

Diagnotic Pathology Center, International University of Health and Welfare (IUHW), Japan

Summary: Pathology of PNEN (WHO Classification 2010) includes well differentiated neuroendocrine tumor (NET), which is divided into NET G1 and G2, and poorly differentiated neuroendocrine carcinoma (NEC), small cell and large cell type, according to the proliferative activities, mitotic index and Ki67 index. Even NET G1 can metastasize and should be considered to be potentially malignant. The PNEN can occur with familial background including MEN1, VHL. Germline mutations are of value for the clinical approach. In the genomic landscape, somatic mutations can occur in NETs with ATRX/ADXX mutations are of good examples. In contrast KRAS mutation can occur in pancreatic adenocarcinoma (PADC).

For cytology, endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is now widely used for the differential diagnosis of PNEN and other tumors including PADC. Genomic analysis has been performed on the cytology specimens successfully.

Two major issues have been brought up for the WHO classification 2010.

(1) Well differentiated NET with high proliferative index should be designated as NET G3 which demonstrates higher SSTR2 and lower proliferative indices comparing to NEC.

(2) Mixed adenoneuroendocrine carcinoma (MANEC) has been reported that both components are of monoclonal origin and has been suggested to follow the staging of PADC.

My presentation will overview current PNEN with molecular and cytopathology and further clarify the current topics which hopefully will be included in the new edition of WHO classification.

ME05

Questions About Digital Pathology, Cytology Automation, and Routine Morphologic Interpretation

David C. Wilbur[1,2]*

[1]Department of Pathology, Harvard Medical School, [2]Clinical Imaging in Pathology, Massachusetts General Hospital, United States

Summary: This program will provide an opportunity for registrants to directly interact with Dr. Wilbur in a semi-formal session designed to allow for maximal group participation. Dr. Wilbur will provide a short introduction to his areas of interest as detailed below. Participants will be encouraged to discuss these areas and ask specific questions which should allow for expanded discussion and interaction. Preregistrants will be asked to submit any specific areas or questions they would like to explore directly to Dr. Wilbur before the conference at dwilbur@partners.org.

Dr. Wilbur's specific interests are in the following areas:

1) Cervical cytology – particularly glandular lesions of the cervix
2) Cervical histopathology – particularly glandular lesions of the cervix
3) Cervical cancer screening algorithms and testing for HPV
4) Cytology automation – particularly cervical cytology automated screening
5) Digital pathology – telepathology and remote consultation
6) 3-dimensional cell analysis

As Dr. Wilbur routinely practices in all areas of cytology, general topics and questions related to cytology laboratory operations, quality assurance, ancillary testing, and the variety of non-gynecologic cytology will also be entertained.

ME06

Ten Tips for IAC Exam Success

*Shirley E. Greening**

International Academy of Cytology, United States

Summary: For four decades, the International Academy of Cytology has administered examinations for cytotechnologists and cytopathologists from over 65 countries. Since the first International Board of Cytopathology exam was given in 1978, almost 800 medical members of the IAC have achieved the title of FIAC. Since 1972, almost 12,000 cytotechnologists have successfully passed the IAC Comprehensive Cytotechnology Examination earning the title CT (IAC).

How did all these cytology professionals achieve this distinction? This session will review and recommend test-taking strategies geared specifically to the IAC exam formats using examples from the microscopic, visual image and written components of each of the exams. On-line preparation and self-test resources will be provided as the session looks at these 'Ten Tips' for exam success:

1. What to study
2. When to Study
3. How exam test items are developed and deployed
4. How exam test items are evaluated: Cytopathology Board Exam
5. How exam test items are evaluated: Cytotechnology Registry Exam
6. Test-taking Strategies: Microscopic Component – the 'ABCD Formula'
7. Test-taking Strategies: Visual Image Component
8. Test-taking Strategies: Written (Multiple Choice) Component
9. Can I review my exam performance?
10. If at first you don't succeed…….

Luncheon Seminars

LS-B-1

Performance of BD SurePath™ and ThinPrep® in the English Cervical Screening Program

Mina Desai[1], *John H.F. Smith*[2]

[1]Editor in Chief, Cytopathology (2015), Head of the Service, Manchester Cytology Center (retired), [2]East Pennine Cytology Training Center, United Kingdom

The advisory Committee for Cervical Screening in UK recommended introducing LBC in England & Wales in 2003. This recommendation came after careful evaluation of ThinPrep® and BD SurePath™ technologies within 3 designated pilot sites in England. U.K. was fully converted to LBC by 2008.

The national statistics from England, Wales & Scotland since the introduction of LBC have shown the clear benefit of LBC with increase detection of HSIL and above and increase in PPV value. The inadequate rate is drastically reduced since the introduction of LBC (reduced to 2.5% from 10%). The performance data comparing 2 LBC technologies are showing interesting differences in the HSIL and above pick up rates and PPV value. These data will be presented.

When comparing the different LBC systems, the majority of the world literature on this subject has concentrated on direct comparisons of the systems with regard to clinical effectiveness. This approach fails to address the practical and technical issues related to LBC systems in linking these technologies with ancillary techniques e.g. HPV and other molecular tests. The author will discuss these differences in the presentation.

LS-B-2

A National Evaluation of Cervical Histology after 15 Years of Routine Liquid-Based Cytology and Computer-Assisted Cytology Reading in Denmark

Jesper Bonde

Molecular Biology Lab Department of Pathology, Hvidovre Hospital, Denmark

Denmark is a high incidence country for cervical cancer, and as such detection by cytology based screening is pivotal for the effectiveness of the national preventive effort. Since 2012/3 only liquid based cytology has been allowed, and conventional cytology is completely out-phased. We have compared the sensitivity and specificity of liquid-based cytology (LBC) and computer-assisted reading for BD SurePath™/BD FocalPoint™ and ThinPrep® with those of manually read conventional cytology in routine cervical screening in four Danish laboratories located across the country. Using data from five nationwide registers, technological phases were identified by slide preparation, reading technique, and triage of borderline cytology. Trends in the detection of cervical intraepithelial neoplasia (CIN) were an indicator of the technology's relative sensitivity, and trends in false-positive tests an indicator of relative specificity.

At 23–29 years, BD SurePath™/BD FocalPoint™ statistically significantly increased the detection of ≥CIN3 by 85% compared with manually read conventional cytology. At 30–44 years, the increase with BD SurePath™/BD FocalPoint™ was 58%. BD SurePath™/BD FocalPoint™ doubled the frequency of false-positive tests at any age. In a fourth laboratory using manually read conventional cytology until national discontinuation, no such trends were seen.

In conclusion, this study confirmed that modern cytology technologies have not been neutral with respect to the detection of cervical disease. In essence, BD SurePath™-based technology increased the sensitivity of cytology for high-grade cervical lesions compared to other cytology, predominantly in women below 50 year of age.

BD-1

BD The BD FocalPoint™– Principles of Operation, Data Supporting Its Use and Experience in a Hospital Laboratory Setting

David C. Wilbur

Massachusetts General Hospital/Harvard Medical School, United States

The BD FocalPoint™ is an FDA-approved primary screening device for cervical cytology specimens. Its unique approach to slide evaluation includes hierarchical scoring of each slide within a set and individual field of view on each slide, which allows for rank ordering of slides and fields of view from highest to lowest probability of containing abnormality. This feature forms the basis for slide set profiling, quality control, and guided screening functionalities. The session will detail these features and the overall operation of the device. In addition, data generated from long term clinical use in a hospital laboratory will be presented showing its actual performance in its intended uses.

BD-3

HPV Tsting in BD SurePath™ – Clinical Use Requires Robust Validation – Experience in a Hospital Laboratory

David C. Wilbur

Massachusetts General Hospital/Harvard Medical School, United States

HPV testing is an integral component of cervical cancer screening, in roles as triage of equivocal cytology results and as a component or stand-alone test in primary screening. In these applications, HPV testing must be robust as the large number of cases using the test would affect overall performance if test operating characteristics were not in line with current evidence-based guidelines. As HPV testing platforms using BD SurePath™ collection media have not to date been approved by the FDA, extensive validation is necessary in each clinical laboratory using this collection method. This session will report the methods and results of successful validation in a hospital clinical laboratory.

LS-D-1

Preliminary Results of Anal Cytology Screening Among HIV-Infected MSM

Jen-Fan Hang

Department of Pathology and Laboratory Medicine, Taipei Veterans General Hospital, Taiwan

After briefly introducing the current guideline on anal cytology screening among HIV-infected men who have sex with men (MSM), we will share our experience on initiation of anal cytology screening in our institute. The talk will cover sampling issue, cytology interpretation, and high-risk HPV testing on anal samples.

LS-D-3

Cytomorphologic Comparison of BD CytoRich™ Liquid-Based Preparations and Conventional Smears for Non-Gynecologic Cytology

Han Suk Ryu

Department of Pathology, Seoul National University Hospital, Korea

Liquid-based preparation (LBP) offers several advantages over conventional smear including enhanced sample quality and increased diagnostic accuracy, mainly because it provides a better cytomorphologic picture and specimen procurement is simple with fully automated processing and staining methods. In addition, it requires less interpretation and screening time for cytopathologists as fewer slides are needed, and the cells are limited to a smaller area on a clear background with excellent cellular preservation. Although SP procedure for cytologic examination is one of the most commonly used LBP methods and both show considerable diagnostic accuracy, it is necessary to recognize that samples processed with the SP technique exhibit several cytomorphologic alterations compared with the conventional smear such as artificial cellular shrinkage or nuclear membrane irregularity, can often lead to misdiagnosis. Cytologic tests have been widely employed to determine presence of cancer cells in several body cavities including pleura and cerebrospinal space etc. Therefore, distinct cytomorphologic characteristics that must be recognized to avoid misinterpretations of non-gynecologic cytology.

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Cervix 1

O-001

See and Treat Strategy of DNA Quantitative Analysis Assist High Grade Squamous Intraepithelial Lesions

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Objective: To investigate the meaning of new see and treat strategy, that DNA quantitative analysis that is used for High grade Squamous Intraepithelial Lesions.

Methods: Select 774 cases recruited cytology HSIL were randomly divided into study group and control group; Study group on the basis of colposcope diagnosis, application of DNA quantitative analysis, after the patients' informed consent, The colposcope examination line above HSIL pathological changes and the DNA quantitative analysis was abnormal cervical loop electrosurgical excision procedure, The patient's biopsy for pathological examination, diagnosis and treatment once completed, this is the new see and treat strategy; The control group using traditional see-and-treat strategy, Compare the difference of the two methods in the diagnosis and treatment of HSIL coincidence rate, missed diagnosis and transition treatment.

Results: Study Group diagnosis rate of 88.4% and 66.7%, higher than the control group, the difference was statistically significant $P < 0.01$; study group overtreatment rate of 5.8%, lower than the control group10%, but the difference was not statistically significant $P > 0.05$; the study group misdiagnosis rate of 5.8%, lower than the control group 23.3%, the difference was statistically significant $P < 0.01$.

Conclusion: Patients with Liquid based cervical cytology for HSIL, quantitative Analysis of DNA ploidy abnormalities, suspected highly lesions by colposcopy examination, Such patients using see and treat strategy are more appropriate.

Disclosure of Interest: None declared.

O-002

Biofunctional Evaluation of Radiosensitivity of Cervical Carcinoma by Micronuclei Count and DNA Fragmentation Assay in Exfoliated Cervical Epithelial Smears

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Introduction: Cancer cervix is a leading cause of cancer death among women and much report in the late stages of disease wherein radiotherapy is the treatment of choice. Intrinsic radiosensitivity of the tumor is one of the most important and independent determinant of both local control and patient survival response to radiotherapy. Micronuclei assay is a rapid and simple means of cytogenetic evaluation of cellular radiosensitivity that helps in deciding the probability of recurrence and the necessary adjuvant treatment. Micronuclei (MN) are extranuclear cytoplasmic bodies, induced in cells by radio therapeutic agents that bring about apoptotic death of cancerous cells by fragmentation of DNA.

Objective: Evaluation of micronuclei counts and DNA fragmentation assays in exfoliated cervical epithelial cells in cervical cancer patients to identify good responders to radiotherapy.

Material and Methods: Pre and post radiotherapy cervical scrape smears from thirty patients of carcinoma of cervix were evaluated for micronuclei counts. The cervical cells from 15 of these patients were subjected to DNA fragmentation assay.

Results: Majority of the patients were in 5th decade and the most common presenting symptom was post menopausal bleeding. Patients were predominantly of FIGO stage III B and showed increasing micronuclei counts with increasing stage. Post radiotherapy there was a significant increment of micronuclei counts with an increment of 183.98% for the number of cells with MN and 174.05% for the total number of micronuclei. The DNA fragmentation assay in majority of the cases showed ladder formation and confirmed the apoptotic death of tumor cells due to radiotherapy.

Conclusion: The micronuclei assay by scrape smear cytology and the DNA fragmentation assay that indicates cell death induced by apoptotic pathway will help predict radiosensitivity and identify individual cervical cancer patients who are good responders to radiotherapy. The study will be of a great benefit to note the personalized response to therapy which is looked forward.

Disclosure of Interest: None declared.

O-003

Transcriptome Analysis of Cervical Cancer Disease Progression

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[1]Memorial University, [2]Eastern Health, Canada

Objectives: We hypothesized that analysing global genome expression of endocervical cells representative of each stage of HPV-related disease progression would elucidate novel molecular processes, biomarkers and therapeutic targets.

Materials and Methods: Transcriptome analyses were performed on normal human endocervical (HEN) cells, HPV-16 infected HEN (H16) cells, tumour forming H16 (H16T) cells and chemoresistant H16T (H16R) cells using Affymetrix microarrays, Significantly up- and down-regulated genes were identified using BRB arraytools. Functional gene clustering of differentially expressed genes, verified using qRT-PCR, were performed using DAVID. RNA from 88 cervical Pap specimens in SurePath medium was analyzed for expression of the most prominent differentially expressed genes.

Results: 358 genes were down-regulated in H16, H16T and H16R cells relative to HEN cells. H16 cells up-regulated a unique set of 59 genes. A unique set of 136 genes and 91 genes were up-regulated in H16T and HEN cells, respectively. H16 and H16R shared 7 genes, 4 of these were up-regulated and 3 of them were down-regulated. Down-regulated genes shared by H16, H16T and H16R were involved cell-cell and cell to extracellular matrix adhesion and degradation. H16 up-regulated genes were involved in chemokine signalling, inflammatory response and cell cycle. Genes expressed in the H16T cells represented cAMP signalling, Melanoma, Inflammation and response to stimuli. Chemoresistant H16R cells expressed genes involved in metastasis, differentiation, ECM degradation, cell junction integrity, migration and invasion. As expected, HPV E7 mRNA levels are significantly lower in Pap negative and LSIL as compared to HSIL ($p = 0.048$). Keratin 6A (KRT6A) mRNA levels are lower in negative and LSIL as compared to HSIL ($p = 0.0018$). Correspondingly, HPV 16 and 18 E7 mRNA levels correlated well with KRT6A levels ($p = 0.045$). MX dynamin like GTPase 1 (MX1, aka Myxovirus influenza resistance 1) mRNA increased in LSIL as compared to normal cells but then decreased in HSIL ($p < 0.0001$).

Conclusion: Cervical cancer progression is associated with down-regulation of a common set of genes involved in cell adhesion and extracellular matrix breakdown, but each individual step leading to malignancy recruits unique sets of genes specific to that stage. At least two of these differentially expressed genes are correlated with diagnosis.

Disclosure of Interest: None declared.

O-004

Immunocytochemical Detection of Raf Kinase Inhibitor Protein and Human Papillomavirus Profiling of Normal and Abnormal Cervical ThinPrep Samples

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Objectives: This study investigates the potential value of Raf kinase inhibitor protein (RKIP) as a marker of normal squamous cells in ThinPrep slides. RKIP was evaluated for its ability to distinguish between normal and abnormal cervical cells samples in the context Human papillomaviruses (HPV) infections.

Materials and Methods: 316 ThinPrep samples were taken from women with normal and abnormal cervices. ThinPrep slides were Papanicolaou stained and reported. Residual samples were used for RKIP immunostaining and HPV PCR based sequencing.

Results: RKIP expression was seen in both nuclei and cytoplasm in 83.7% of samples. RKIP expression was highest (84.6%) in samples with high-grade squamous intraepithelial lesion (HSIL) or worse diagnosis, expression decreased in low-grade squamous intraepithelial lesions (73%) and was least in samples with normal cytology, $p = 0.0023$. 74% of HPV infected ThinPrep samples were immunopositive and 67% of samples that did not harbor HPV were also immnopositive, $p = 0.414$. Sensitivity and specificity of RKIP were 84.6% and 34.6%, respectively, for the detection HSIL or worse samples.

Conclusion: The study showed that RKIP expression may be of some value as a marker for abnormal cervical cells. Combined RKIP expression and HPV testing could improve the identification of samples with abnormal cytology reports.

Disclosure of Interest: None declared.

O-005

Comparison of Interpretation of p16/Ki67 Dual Immunocytochemical Staining Results on Cervical Smears in Three Cytopathology Laboratories Participating in Slovenian Cervical Cancer Screening Program Zora

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Background: p16/Ki67 dual immunocytochemical staining (ICS) has been confirmed as sensitive and specific triage test for HPV positive women and as a potential screening test for cervical cancer. Literature indicates this test as easy to interpret, however, our experience was different. Therefore we decided to study concordance of p16/Ki67 results between 3 Slovenian cytopathological laboratories involved in the national screening programme.

Methods: In 129 women referred to colposcopy, cervical smears were obtained for p16/Ki67 dual ICS (CINtec PLUS test, Roche). Each ICS was evaluated blindly in 3 laboratories by screener and cytopathologist who only had informal training. Results were reported as positive, suspicious, negative or inadequate. After primary evaluation all evaluators underwent an extensive learning led by an expert, including discussion of difficult cases. Difficult cases were revaluated blindly in all 3 laboratories and results compared to primary evaluation. Agreement among laboratories was evaluated using Cohen's Kappa in SPSS 16.0. Sensitivity and specificity for CIN2+ were also calculated.

Results: 79/129 (61.2%) cases were identified as difficult due to discordance between positive and negative results, number of positive cells/groups, or number of inadequate or suspicious cases. After extensive learning the rate of positive results increased from 45.2% to 55.3% and the rate of negative results from 41.6% to 42.9%. Suspicious and inadequate results decreased (from 10.6% to 1.6% and from 2.6% to 0.3%). Exact agreement among 3 laboratories increased from 61.2% (Kappa 0.60–0.74) to 89.1% (Kappa 0.84–0.88). Sensitivity for CIN2+ increased in all three laboratories (from 78.2–85.5% to 83.6–89.1%). In two laboratories the increase of sensitivity was not associated with the loss of specificity (in one laboratory the specificity remained 67.7% and in the other increased from 48.5% to 60.0%), while in one laboratory there was a small decrease in specificity (from 68.9% to 67.1%).

Conclusion: Extensive learning process led by an expert and discussing difficult cases proved to be necessary for accurate interpretation of p16/Ki67 dual ICS in laboratories involved in our organised screening program. The training also resulted in increased sensitivity of the test for CIN2+ in all 3 laboratories without substantial loss of specificity.

Disclosure of Interest: None declared.

O-006

The Study of Consistency Analysis between Liquid-Based Cytology Asatypicalsquamous Cell Which Cannot Excludehigh Grade Squamous Intraepithelial Lesion (ASC-H) and Follow-Up Cervical Biopsy

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Objective: The objective of this study was to evaluate the consistency between liquid-based cytology as ACS-H and follow-up tests assessment, included colposcopy, endocervical curettage evaluation and cervical biopsy. Of these 310 cases in total were had histological follow-up results.

Materials and Methods: A total number of 102,300 liquid-based cervical cytology samples were collected from the beginning of 2012 through the end of 2015 in two different institutions of Guangdong (49400) and Guangxi (52900) Province in China, and only 513 cases were diagnosed as ASC-H in total. Of these 310 cases cervical biopsy evaluation were performed and histological follow-up results were available. Cases with previous history of any type of intraepithelial lesion were excluded.

Results: The reporting rate of ASC-H was 0.63%(311/49400) and 0.38%(202/52900) in the institution of Guangdong and Guangxi Province in China, respectively. The follow-up cervical biopsy rate was 57.32%(178/311) and 65.35%(132/202) in Guangdong and Guangxi Province, respectively. A total number of 310 patients were included in this study. Data in the institution of Guangdong Province demonstrated that approximately 48.31% (86/178) patients were diagnosed by colposcopy and cervical biopsy as Cervical intraepithelial neoplasia (CIN) 2, 3 or cancer in initial diagnosis. 38 cases were CIN 2, 42 cases were CIN 3, 5 cases were squamous cervical cancer, 1 case was adenocarcinoma of the cervix. Similarly, date in the institution of Guangxi Province showed that approximately 55.30% (73/132) patients were diagnosed histological as cervical intraepithelial neoplasia (CIN) 2, 3 or cancer in first evaluation. 31 cases were CIN 2, 34 cases were CIN 3, 6 cases were squamous cervical cancer, 1 case was adenocarcinoma of the cervix and 1 case was adenocarcinoma of the endometrium. In Guangdong Province, the average age of different ASC-H biopsy results were statistical significance ($F = 3.485$, $P = 0.005$) while there were no difference in Guangxi Province ($F = 0.673$, $P = 0.644$). After compared the different distribution of ASC-H biopsy results, there were no statistical significance ($P > 0.05$) between these two regions.

Conclusion: It was still cannot consider there was a different distribution of ASC-H biopsy results between Guangdong and Guangxi Province in China. It may be related to the similar medical diagnostic level and distribution types of Gynecological diseases. The new evidence in this study indicated that a change for management of women with abnormal cervical cancer screening tests especially diagnosed as ASC-H may be needed.

Disclosure of Interest: None declared.

O-007

Stratified Mucin-Producing Intraepithelial Lesion (SMILE) of the Cervix: Subtle Features of a High-Grade Lesion Not to Be Missed

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Objectives: SMILE is an uncommon premalignant lesion of the uterine cervix characterized by stratification, mucin production and absence of classic gland formation. Literature on the cytologic features, HPV genotype and clinical implications is limited. We here examined the cytologic features of SMILE in contrast to AIS using cases selected for their SMILE-dominant pattern and determined the HPV genotypes in a series of cases with SMILE.

Methods: Natural language searches of the laboratory information systems of 2 tertiary care centers were performed with combinations of 'SMILE', 'adenocarcinoma', 'adenosquamous', 'stratified' and 'in-situ' (years 2000–2014). Excision specimens and immediately preceding Pap tests were retrieved for cases without concurrent invasive carcinoma. Excisions were grouped according to the estimated lesional proportion of SMILE (≥50% *vs.* <50%). Pap tests were assessed by 4 cytopathologists for 5 architectural, 16 cellular and 5 background features. AIS controls were selected based on minimal/absent invasion and preparation type (2 SurePath® (SP), 2 ThinPrep® (TP), 1 direct smear (DS)). Observers were blinded with respect to the controls. 4 observers x 5 cases accounted for a total of 20 (100%) observations per feature. HPV genotyping was performed on formalin-fixed paraffin-embedded excisions (cobas® HPV Test (Roche)).

Results: 13 SMILE cases were identified. Median patient age was 33 years; range 23–51. SMILE was the dominant pattern (≥50%) in 6 cases and was represented in 5/6 preceding Pap tests (3 SP, 1 TP, 1 DS). Cytologic features of SMILE and AIS showed common overlap. No feathering was detected in SMILE, whereas this feature was recognized in 35% of the observations for AIS. Nucleoli were mostly absent in SMILE (55%), whereas small or prominent nucleoli were detected in AIS (65%). Other differences (>20% threshold) included the degree of group organization and crowding, distinctness of the cytoplasmic border, nuclear shape and chromatin structure. 12 cases were available for HPV genotyping. HPV18 was detected in 7, HPV16 in 4 and other types in 4 cases. Multiple HPV types were detected for 3 cases.

Conclusions: SMILE is a lesion that may be best considered as AIS variant for cytologic, etiologic and management purposes. The cytologic features overlap with AIS, but may be more subtle and may be interpreted as atypical glandular cells. Interpretation as reactive endocervical cells should be avoided as it may lead to delays in the diagnosis of a lesion less likely to be identified by colposcopy. HPV testing may play a role in facilitating SMILE detection.

Disclosure of Interest: None declared.

O-008

Cervical Adenocarcinoma with Stromal Micropapillary Pattern (SMP)

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Adenocarcinoma with a stromal micropapillary pattern (SMP) has been described in various organs, but not in the uterus. We encountered a case of uterine cervical carcinoma with SMP. A54-year-old Japanese woman was referred to the hospital with abnormal vaginal bleeding. The cervical cytodiagnosis was adenocarcinoma with features resembling serous adenocarcinoma. Cervical cytology showed many small clusters of tumor cells, present in up to two or three layers, composed of atypical cells with markedly increased nucleus: cytoplasm ratios. A radical hysterectomy with bilateral adnexectomy and retroperitoneal lymph node dissection was performed. Microscopically, the tumor was composed predominantly of adenocarcinoma with SMP. The outer surface of the SMP cell clusters showed membranous expression of mucin-1 (MUC-1). Many lymph node metastases were detected. The tumor was diagnosed as a cervical adenocarcinoma with SMP and coexistent squamous cell carcinoma in situ. The pathology was classified as T1b1N1M1, stage IVB. The patient underwent postoperative adjuvant chemotherapy and is without local recurrence or distant metastasis 48 months after the operation. To the best of our knowledge, this is the first reported case of cervical adenocarcinoma with SMP.

Disclosure of Interest: None declared.

O-009

Cervical Mucinous Adenocarcinoma of Gastric Type; Cytological Findings with Immunocytochemistry

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Objectives: Cervical mucinous adenocarcinoma of gastric type is a newly recognized histological type listed-up in the 4th edition of WHO Classification with the re-categorized entity of endocervical adenocarcinoma of usual type (UEA). There only are a few reports which mention about the cytological findings of gastric type. We aimed to find the cytological characteristics and significance of immunocytochemistry in liquid-based cytology (LBC) (SurePath™, BD).

Materials and Methods: Cytological material of 35 cases of cervical adenocarcinomas checked by LBC and diagnosed histo-

logically during 4 years until 2011 were reviewed. The data of immunocytochemistry were used only those of semi-routinely processed materials, not those of retrospective examination.

Results: There are 19 cases of usual type followed by 10 cases of gastric type including one of minimal deviation adenocarcinoma (MDA), 4 cases of AIS and each 1 case of villoglandular and intestinal types. The cytological findings of gastric type comparing to those of UEA are rather plain sheet formation with distinct honeycomb pattern, loss of nuclear polarity with anisonucleosis even in the plain sheets, and fine chromatin pattern with no relativity to nuclear atypia.

In 19 cases of all types were checked with immunocytochemistry for $p16^{INK4A}$, and/or MIB1. Among 9 cases of UEA and 1 of villoglandular type, 8 were positive for $p16^{INK4A}$ (88.9%). All 8 cases of gastric type performed for the immunocytochemistry were negative for $p16^{INK4A}$. Four of 5 cases performed for HIK1083 (marker of gastric type mucin, Kanto Kagaku) showed positive immunoreactivity. MIB1 rate assessed in cluster showing the highest labeling rate is more than 30% even in the case of MDA and the most were above 75%. This is significantly higher than that of control group (mostly 0%, highest 27%).

Conclusion: Immunoreactivity for $p16^{INK4A}$ is a useful marker for a diagnosis of adenocarcinoma as is so in that of high grade squamous cell lesions for a dense staining characteristic and high positive rate. Gastric type adenocarcinoma, however, is negative for the marker having a relevance to non-HPV etiology for its carcinogenesis. This should be recognized in cytological examination of the glandular lesions. Combining the cytological morphology and the data of immunocytochemistry with panel of mentioned antibodies LBC may introduce a correct diagnosis even to the level of subclassification of histological type.

Disclosure of Interest: None declared.

O-010

Cytological Morphology and Immunophenotype of Gastric Type Adenocarcinoma of the Uterine Cervix

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Background: Gastric-type is a newly identified, important subtype of endocervical adenocarcinoma because of its morphological features and resistance to chemotherapy and radiotherapy, whereas its low cellular atypia frequently disturbs the cytological screening and diagnosis.

Objectives: To reveal and improve the difficulties in its screening and diagnosis, we investigated the cases in our hospital.

Materials and Methods: The symptoms, cytological morphology and immunophenotypes of 9 cases histologically proved to have gastric type adenocarcinoma from 2011 to 2015 were investigated.

Result: Their age ranges 36–85, and FIGO stags was IB1: 5 cases, IIB: 1, IVA: 1, IVB: 2. Four cases started with cancer screening and others had some symptoms (genital bleeding: 2 cases, abdominal pain: 2, abnormal discharge: 1). The cytological diagnoses were AGC-NOS: 1, AGC-FN: 2, Adenocarcinoma: 1 in the screening group, and NILM: 2 cases, AGC-NOS: 2, AGC-FN: 2, Adenocarcinoma: 2 in the symtomatic group. Both cases of NILM had abnormal endometrial cytology, coming from atypical cervical glands. There were few clusters of atypical glandular cells in AGC cases, and just 1 of 4 AGC and 1 of 3 Adenocarcinoma cases showed voluminous cytoplasm. In hystology, the stromal invasive cases demonstrated clear eosinophillic, voluminous cytoplasms and distinct cell borders. Immunophenotypes were P53 (+)/(–): 3/5 cases, $P16^{INK4a}$ (+)/(–): 0/8, ER (+)/(–): 0/7, HIK1083 (+)/(–): 1/3. High risk HPV-DNA was examined in 1 of 9 cases and resulted in negative.

Conclusion: In our cases, their symptoms, cytological morphology and immunophenotypes were so various, however negative P16 and ER might be characteristic. Especially negativity of P16 was a distinct feature from usual-type of endocervical adenocarcinoma. High risk HPV is not involved in the oncogenesis of gastric-type and it is not detectable in high risk HPV-DNA test. Thus, a case with cytological AGC and negativity in high risk HPV-DNA should proceed to further examination with biopsy. Furthermore, even NILM requires repetitive cytology, if there are some clinical symptoms found in gastric type adenocarcinoma.

Disclosure of Interest: None declared.

Endometrium

O-011

Utility of Endometrial Aspiration Cytology for Screening Postmenopausal Women for Endometrial Malignancies

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Objective: Endometrial aspiration cytology (EAC) is an acceptable and valuable diagnostic procedure for screening the endometrial status. Objective of this study is to know the utility of this procedure as a screening procedure for detection of endometrial malignancies in postmenopausal women with abnormal uterine bleeding (AUB).

Methods: Endometrial aspiration obtained using 5F infant feeding tube attached to 20cc disposable syringe. Endometrial aspiration material was smeared directly on to three clean glass slides. One smear was wet fixed for papanicolau staining and the remaining slides were air dried for Giemsa stain. Smears were reviewed for cytomorphological findings and were correlated with the histopathological findings.

Results: Age of the patients ranged from 45 to 70 years. In our study, the sensitivity and specificity in diagnosing malignancy on aspiration cytology were 88.5% and 100% respectively.

Conclusions: Endometrial aspiration is an effective, useful and a minimally invasive procedure. With an experienced cytologist, it can be used routinely for the screening of postmenopausal women with AUB for endometrial malignancies, provided all the points of discrepancies are taken care of.

Disclosure of Interest: None declared.

O-012

Direct Endometrial Brush Cytology in Asymptomatic Patients Reffered for Endometrial Evaluation

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Objective: The aim of this study was to analyse the use of endometrial brushing cytology in asymptomatic patients reffered to the hospital for endometrial evaluation and its possible role in decision making of the necessity of further invasive procedures such as curretage.

Materials and Methods: In 220 patients without or with wery scant signs of uterine bleeding endometrial brush cytology was performed. Endometrial smears were stained with Papanicolaou staining procedure. Adequacy of material was recorded as inadequate: without endometrial cells and adequate: with endometrial cells present for analysis. Cytology results were classified as: negative, atypical and positive (malignant). Results were compared with histological results or with follow up data.

Results: Inadequate samples were recorded in 14% (N = 30) of the cases. Out of the 190 adequate samples in 95.3% (N = 181) cytology result was negative, in 1.6% (N = 6) was atypical and in 3.1% (N = 6) was positive. Endometrial curretage was performed in 32 (17%) patients and follow up data were available for 67 patients. Out of the six cytology positive patients malignant lesion was confirmed in five, and data from one patient was not available. Out of the three atypical cytology finding all three were histologicaly negative. In the patients with negative cytology only one was found to have malignant lesion and it was extranodal non-hodgkin endometrial lymphoma. From these data we calculated the sensitivity of endometrial brush cytology for asymptomatic patients and it was 83.3%, specificity was 100%, positive predictive value was 100% and negative predictive value was 98.9%.

Conclusion: Direct endometrial brush cytology is highly sensitive and specific. It represents a valuable diagnostic tool in clinical management of asymptomatic patients reffered for endometrial curretage, allowing delay or diminish the need of this invasive and expensive procedure.

Disclosure of Interest: None declared.

O-013

New Diagnostic Reporting Format for Endometrial Squash Cytology Based on Cytoarchitecture

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Introduction: Endometrial carcinoma is the third most common cause of cancer related deaths of women, behind ovarian and cervical cancer. Endometrial cytology can be an early, cheap and rapid method for its screening and diagnosis. But compared to cervical cytology, it is considered unreliable and less reproducible diagnostically. This is because of lack of a proper reporting format, clear definition of specimen adequacy and diagnostic criteria for establishing benign and malignant lesions. But over the years the importance of endometrial cytology in diagnosis and management of endometrial carcinoma is becoming established. Therefore this study was undertaken to develop a new reporting format for endometrial cytology that would standardize the diagnostic criteria. that reflect the cytoarchitecture and the conventional criteria (background, atypia of cells, or cell clumps).

Materials and Methods: This study was carried out over a period of two years in Department of Pathology and Obstetrics & Gynecology, S.C.B. Medial College, Cuttack. The proposed format investigated 200 cases of endometrial squash cytology obtained from patients undergoing dilatation and curretage. The clinical informations, investigation and imaging findings were collected and correlated. All the cytologic smears were analysed according to new reporting format considering the cytoarchitecture and conventional criteria. Lesions were classified as negative, suspicious or positive. And these findings were correlated with histopathology and were recorded as cytologically underestimated or overestimated in comparison to histopathological diagnosis.

Results: Out of the 200 cases studied, 164 could be correlated well with histopathology. Of the remaining 36 cases, 4 were reassessed and rendered unsatisfactory. Among the rest 32 cases, 20 cases which were underestimated were subsequently diagnosed having endometrial carcinoma or its precursor lesions on histopathology. Remaining 12 cases which were overestimated were all reassessed as not needing treatment.

Conclusion: The reporting format for endometrial cytology may improve the diagnostic accuracy and reduce the number of patients treated inappropriately. This is useful for the proper cytological assessment of endometrial lesions and can classify them into negative, suspicious or positive which can be of great benefit to patients.

Disclosure of Interest: None declared.

O-014

Feasibility Study and Histological Correlation of the New Classification of Endometrial Cytology Using Liquid Based Cytology

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Objective: In Japan, endometrial cytology has been widely used as screening tool. While a 3-tier reporting system, consisting of 'negative', 'suspicious', and 'positive' categories, has been used, traditionally, a more descriptive system, the New Terminology in Endometrial Cytology (NTEMC), which is based on the Bethesda System for uterine cervical cytology, was introduced recently. The objective of this study was to validate the NTEMC criteria.

Methods: Sixty eight endometrial cytology specimens were taken from the hysterectomized uterus operated during 2012 February and 2014 January. Liquid based cytology (LBC) specimens were made and stained by Papanicolaou method. Each specimen was classified according to the diagnostic category of the NTEMC by five of the members in our department. The diagnostic category, chosen by the majority of the examining members, was recorded. Each cytological diagnostic category was correlated with histological diagnostic category.

Results: Negative, ATEC-US, ATEC-A, endometrial hyperplasia, atypical endometrial hyperplasia, and malignancy was selected as the cytological diagnostic category for 42 (61.8%), 5 (7.4%), 2 (2.9%), 2 (2.9%), 1 (1.5%), and 26 (23.5%) specimens, respectively. Cytological category was significantly correlated with histological category ($p < 0.0001$). Three out of 5 cases in ATEC-US category were correlated with benign histology (60%). In contrast two out of 2 cases in ATEC-A category was correlated with malignant histology (100%). Sensitivity, specificity, positive predictive value, negative predictive value was 81.8%, 97.8%, 94.7%, 91.8%, respectively. Kappa value for agreement of five members was 0.32.

Conclusion: The ATEC category in the NTEMC system worked well by using LBC.

Disclosure of Interest: None declared.

O-015

Cell Block Preparation of Endometrial Specimen Does Not Improve the Sensitivity of Endometrial Cytology for Diagnosis of Endometrial Cancer

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Objectives: In Japan, endometrial cytology is widely used as a supplementary tool for diagnosis of endometrial cancer. However, it was reported that the sensitivity of endometrial cytology was 80–85%. In this study, we investigated whether cell block preparation may improve the sensitivity of endometrial cytology.

Materials and Methods: Endometrial scraping cytology, cell block specimens and aspiration biopsy from 19 cases of endometrial cancer histologically confirmed with the examination of surgically resected uterus were analyzed.

Results: Median age of cases: 55 years old (33–63). Definitive pathological diagnosis of surgically resected uterus: 2 atypical endometrial hyperplasia, complex (10.5%); 12 Grade 1 (63.2%); 3 Grade 2 (15.8%); 2 Grade 3 (10.5%). Endometrial cytology: 11 positive (57.9%); 7 suspicious (36.8%); 1 negative (5.3%). Cell block preparation of endometrial specimen: 7 positive (36.8%); 4 suspicious (21.1%); 5 negative (26.3%); 3 unsatisfactory (15.8%). Endometrial aspiration biopsy: 19 positive (100%).

Conclusion: Cell block preparation of endometrial specimen does not improve the sensitivity of endometrial cytology for diagnosis of endometrial cancer. Endometrial cytology might be useful in the particular cases those are suspected of endometrial cancer clinically and are not possible to diagnose endometrial cancer with endometrial aspiration biopsy. The results of ongoing prospective study of JAOG (Japan Association of Obstetricians and Gynecologists) on cytological screening for endometrial cancer using liquid based cytology are awaited.

Disclosure of Interest: None declared.

O-016

Potential Roles of PSMB9/b1i and CAVEOLIN 1 Define New Targets for Uterine Leiomyosarcoma Therapy

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Background: Although the majority of smooth muscle neoplasms found in the uterus are benign, uterine leiomyosarcoma (LMS) is extremely malignant, with high rates of recurrence and metastasis. We earlier reported that mice with a homozygous deficiency for PSMB9/b1i, an interferon (IFN)-g-inducible factor, spontaneously develop uterine LMS. The IFN-g siganal pathway is important for control of tumor growth and invasion and has been implicated in several malignant tumors.

Aim: It is necessary to analyze risk factors associated with human uterine LMS, in order to establish a diagnostic biomarker and a clinical treatment method.

Methods and Results: In this study, experiments with mouse uterine tissues and human clinical materials revealed a defective PSMB9/b1i expression in human uterine LMS that was traced to the IFN-g signal pathway and the specific effect of JAK-1 somatic mutations on the PSMB9/b1i transcriptional activation. In addition, CAVEOLIN1 (CAV1) expression was decreased in the normal myometrium, whereas it was markedly expressed in uterine mesenchymal tumors. Furthermore, analysis of a human uterine LMS cell line and human clinical materials clarified the biological significance of PSMB9/b1i in malignant myometrium transformation and tumor senescence, thus implicating PSMB9/b1i as an anti-sarcomagenic candidate.

Conclusion: PSMB9/b1i and CAV1 differential expressions may be potential diagnostic biomarker for human uterine mesenchymal tumors, especially human uterine LMS. This role of PSMB9/b1i as a tumor suppressor may lead to new diagnostic biomarker and therapeutic target molecule in human uterine LMS.

Disclosure of Interest: None declared.

O-017

Ascites Cytology Alone Is Not Sufficient for Peritoneal Metastasis Assessment in Ovarian Carcinoma Staging Laparotomy

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Background: Peritoneal metastasis is an independent prognostic factor of ovarian carcinoma. Exfoliative cytology of ascites or peritoneal lavage and swabbing has been widely used for detection of peritoneal metastasis in Japan. International guidelines recommend additional random peritoneal biopsies from each of the abdominal sites, allowing sub-diaphragm scraping cytology as an acceptable alternative in order to complement false-negative peritoneal cytology.

Objectives: The aim of this study was to assess the efficacy of peritoneal cytology of ascites/lavage and peritoneal swabbing with a cotton swab to detect peritoneal metastasis during staging laparotomy for ovarian carcinoma.

Methods: We conducted IRB-approved study to analyze ovarian carcinoma cases between January 1, 2004 and December 31 2010. 138 ovarian carcinoma cases without neo-adjuvant chemotherapy who were performed peritoneal cytology of ascites/lavage and/or swabbing by cotton swab during the laparotomy were analyzed. The results of peritoneal cytology and the clinical aspects of each case were assessed by the medical chart. Random peritoneal biopsies in cases without macroscopic peritoneal metastasis were not routinely performed during this period.

Results: In 55 stage I patients, six had malignant peritoneal cytology with the histopathology of four clear cell, one mucinous, and one endometrioid. 103 of 138 (75%) stage I – IV cases had pooled ascites in the peritoneal cavity, and among them, 101 cases were tested by ascites cytology. Clear cell and serous carcinoma were significantly frequently positive for malignant cells; 18 of 25 (72%) for serous, 15 of 25 (60%) for clear cell, 4 of 19 (21%) for endometrioid, 2 of 11 (18%) for mucinous, and 9 of 21 for other histology ($p < 0.02$, Chi-square test). Strikingly, 31% of the cases with pT3b or pT3c tumors with ascites, who were expected to have malignant cytology, resulted in negative. The cases with malignant-cell negative ascites were detected frequently in endometrioid histology, although it was not statistically significant: 2 of 10 (20%) for clear cell, 5 of 23 (22%) for serous, and 2 of 5 (40%) for endometrioid carcinoma. There was no case being upstaged by sub-diaphragm swab cytology.

Conclusion: For staging ovarian carcinoma, cytology of ascites/lavage alone is not enough for detection of peritoneal metastasis. Careful surveillance and biopsies of the peritoneal surface is necessary in number of cases. For sub-diaphragm assessment, collection of peritoneal cells by cotton swab could not detect additional malignancy. We suggest that the devises used for this procedure should be carefully selected.

Disclosure of Interest: None declared.

O-018

Evaluation of Intraoperative Cytology in Diagnosis and Management of Ovarian Tumors

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Introduction: Ovarian neoplasms are a heterogenous group of benign and malignant tumors which can be of epithelial, stromal and germ cell origin. Most of the ovarian tumors cannot be easily distinguished from one another on the basis of their clinical, radiologic or gross features alone. Preoperative cytology can lead to seeding of tumor cells and cause harm. Therefore, intraoperative cytologic interpretation of ovarian neoplasms is both essential and challenging. More so in set ups where facility for frozen section is not available. So this study was done to establish the validity and reliability of cytology in intraoperative diagnosis & management of ovarian tumors and to compare it with histopathology.

Methods: This is a prospective study where materials were obtained from patients undergoing surgery for ovarian lesions after taking informed consent. Multiple imprint, scrape and squash smears were taken from resected ovarian masses and immediately fixed with absolute alcohol. They were stained with hematoxylin and eosin, examined under light microscope, and the findings were reported and compared with subsequent histopathology report.

Results: Total number of ovarian tumors studied was 128. There were 25 (19.53%) cystic lesions, 69 (53.90%) benign and borderline tumors, and 34 (26.56%) malignant tumors. Overall diagnostic accuracy was 85.93% (in 110 out of 128 cases). This data is comparable with the findings of other authors.

Conclusion: Intraoperative cytology is a less expensive, simple and quick method of diagnosis of ovarian tumors. It can allow individualization of treatment, especially in young patients who need conservative surgery in order to preserve fertility. Also it aids in planning for proper management in case of advanced tumors of high grade if detected beforehand.

Disclosure of Interest: None declared.

O-019

Intraoperative Cytology for Ovarian Tumor

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Objective: Intraoperative diagnosis for ovarian tumor, especially solid tumor was occasionally difficult to make a proper diagnosis. In our hospital, we take an intraoperative cytology with conventional frozen histopathology. Elucidate to the effectiveness of intraoperative cytology, we reviewed the intraoperative diagnosis for ovarian tumors, retrospectively.

Materials and Methods: From March 2013 to November 2015, we experienced 58 case of intraoperative diagnosis for ovarian tumor with combing intraoperative cytology. We reviewed intraoperative cytology specimen (PAP, HE and Giemsa staining) from our archives and compared the frozen and permanent histopathological specimen.

Results: All cases were all female and ages are twenty to seventy-eight (average. 51.6 years). Forty eight cases are cystic tumor (Average 52.4 year) and ten cases are solid tumor (average. 48.4 years).

In cystic tumor with solid parts, no useful information was obtained about stromal invasion. But inflammatory cells aggregation and existence of necrosis were related to existence of stromal invasions. In solid tumor, artifacts due to freezing procedures were unable to observe detailed characters of nuclei. But clear nuclear findings were obtained by cytology, especially tumor cells with nuclear grove or immature neuronal cells. Occasionally, few tumor cells were obtained by touch smear methods, but using three staining methods (PAP, HE and Giemsa) were very useful, especially HE specimen. (Discussion and conclusion) In intraoperative diagnosis for ovarian tumor, combining touch smear cytology obtained useful information and anticipated the making more accurate diagnosis.

Disclosure of Interest: None declared.

O-020

Profiling of Human Ovarian Cancer Using Comprehensive microRNA Analysis

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Objectives: Exosomes are small vesicles secreted by most cells. Endogenous microRNAs (miRNAs) play critical roles in many biological processes, including tumor growth, apoptosis and tumor genesis. However, pharmaceuticals and treatments that target them are still in development. Moreover, it is not entirely clear that function of endogenous miRNAs of ovarian cancer exosomes for tumor growth, growth inhibition and invasion. The objective of this study was to clarify the profile of miRNAs in exosomes in culture supernatant and cell lysate of ovarian carcinoma cell lines. Recent reports suggested that exosomal endogenous miRNAs in serum and body fluid are stable. It can be expected to detect new factors of tumor growth, growth inhibition and invasion of ovarian cancer.

Materials and Methods: We used four ovarian cancer cell lines of different histological types such as serous carcinoma (HRA), endometrioid carcinoma (TOV-112D), clear cell carcinoma (HAC-2) and mucinous carcinoma (MCAS), and human ovarian surface epithelial cell line (HOSE). Exosomes were isolated from cell culture media using exoEasy Maxi Kit (QIAGEN). Next, total RNA including miRNA was extracted from exosomal fractions using RNeasy Micro kit (QIAGEN). And then, the concentration and quality of miRNA in total RNAs were analyzed using the bioanalyzer (Agilent). Finally, comprehensive analysis of exosomal and

cell lysate miRNA was performed using human miRNA microarray.

Results: 1. Isolation of exosome was confirmed by detection of CD63 an exosomal protein marker, using a Western blot.

2. Isolation of exosomal miRNA from culture media of ovarian cancer cell lines was confirmed by checking quantification and quality of miRNA with bioanalyzer.

3. miRNA profiling analysis revealed that several miRNAs are differentially expressed (<0.66- or >2-fold) in four ovarian cancer cell lines compared with HOSE.

One miRNA expressed higher, while 99 miRNAs expressed lower in exosomes of all ovarian cancer cell lines than that of HOSE. In addition, 22 miRNA expressed higher, while 16 miRNAs expressed lower in cell lysate of all ovarian cancer cell lines than that of HOSE.

14 miRNAs in exosomes and cell lysate expressed lower commonly, while there was no miRNA that expressed higher commonly.

Conclusion: Analysis method of exosomal endogenous miRNA extracted from culture media of ovarian cancer cell lines was established, and profiles of miRNAs in exosomes and cell lysates of ovarian cancer cell lines of different histological types were clarified.

Disclosure of Interest: None declared.

Molecular Testing and Others

O-021

Assessment of Cytological Specimens Based Molecular Analysis in Advanced Non-Small-Cell Lung Cancer

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Objectives: We investigate the use of anaplastic lymphoma kinase (*ALK*) fluorescence *in situ* hybridization (FISH) and epidermal growth factor receptor (*EGFR*), kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutational testing on cytological specimens in selecting advanced non-small cell lung cancer (NSCLC) patients who are adequate for targeted treatment.

Materials and Methods: A total of 137 non-small cell lung carcinoma (NSCLC) cases of were analyzed by FISH for *ALK* rearrangements. Molecular tests were performed on cytological specimens of 91 fine-needle aspirates, 5 fibreoptic bronchoscopic derived samples and 41 pleural effusions. *EGFR* and *KRAS* mutations were evaluated by quantitative real-time PCR (qRT-PCR) platform combining amplification refractory mutation system (ARMS) primers and TaqMan probes.

Result: Among 137 NSCLCs analyzed for *ALK* FISH, 16 (11.7%, of 137) demonstrated an *ALK* rearrangement. FISH positive cases were all defined as adenocarcinoma (ADC) histologic subtype and the FNA samples showed highest FISH positive rate (13.2%, 12/91). Of the 9 *ALK* FISH positive patients who received crizotinib treatment, 8 (88.9%) patients exhibited tumor regression and the treatment response demonstrated the existence of *ALK* rearrangements detected by FISH in cytological specimens. In addition, 60 (44.8%, of 134) cases carried *EGFR* mutations and 22 patients with *EGFR* sensitive mutations who received gefitinib or erlotinib treatment showed a median PFS of 16.0 months. Mutations of *KRAS* occurred in 8 (6.0%, of 134) cases and this was mutually exclusive from *EGFR* mutation.

Conclusion: *ALK* FISH and *EGFR, KRAS* mutational analyses on cytological specimens are sensitive methods for screening advanced stage NSCLC patients who are adequate for targeted treatment.

Disclosure of Interest: None declared.

O-022

Rapid, Integrated and Fully Automated EGFR Mutation Testing by a Prototype Idylla™ Assay: An Evaluation Study on Lung Cancer Routine Cytological Samples

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Objectives: Ideally, EGFR testing on lung cancer cytology should be carried out in the same center where the patient is diagnosed, such that the most cellular slide can be easily selected from in-house collected cytological material. The Idylla™ EGFR prototype assay is a rapid, integrated and fully automated test running on the Idylla™ fully integrated and automated system, which may easily be adopted on a larger scale by a wider number of pathological centers.

Materials and Methods: Before clinical implementation, we evaluated the lowest limit of detection (LOD) of our laboratory-developed assay in PC9 cells, which harbor a Glu746-Ala750 deletion in exon 19; in H1975 cells, which carry the L858R point mutation; and in A549 cells, which carry the WT EGFR gene. Before clinical implementation, we evaluated the lowest limit of detection (LOD) of our laboratory-developed assay in PC9 cells, which harbor a Glu746-Ala750 deletion in exon 19; in H1975 cells, which carry the L858R point mutation; and in A549 cells, which carry the WT EGFR gene. The limit of detection (LOD) of the Idylla™ EGFR prototype assay was assessed by cell line dilution studies; its performance was evaluated on 46 lung cancer cytological samples (18 mutant and 28 wild type), whose mutational status had previously been assessed by standard techniques, such as length fragment assay (exon 19 del) and Taqman assay (L858R).

Results: The prototype Idylla™ EGFR assay had LOD of 1% mutant allele; in all instances it confirmed the mutant cases (exon 19 del n = 14 and L858R n = 4). In addition, two more mutations

were detected; these included a low abundant exon 19 del and a G719A that was not covered by the standard assay.

Conclusions: These preliminary data showed that the fully automated prototype Idylla™ EGFR assay may serve as a reliable approach, which may be exploited to improve the quality of EGFR community testing.

Disclosure of Interest: None declared.

O-023

Automated PCR Detection of KRAS Mutation Analysis in Endoscopic Ultrasound Fine Needle Aspiration Pancreatic Lesions: A Diagnostic Test Accuracy Study

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Objectives: The use of endoscopic ultrasonography (EUS) has allowed for improved detection and pathologic analysis of fine needle aspirate (FNA) material for pancreatic lesion diagnosis. The molecular analysis of KRAS has further improved the clinical sensitivity of preoperative analysis. For this reason, the use of highly analytical sensitive and specific molecular tests in the analysis of material from fine needle aspirate specimens has become of great importance. The Idylla technology is cartridge based and uses microfluidics processing with all reagents on board. The PCR is real time and uses a fluorophore-based detection system. The post-PCR curve analysis is automated on board the console and the results are presented on screen as either 'No mutation detected' or KRAS mutation detected'. The aim of this study was to validate the Idylla technology on EUS-FNA as a tool to rapidly assess KRAS mutation status.

Materials and Methods: In the present study, 42 specimens from EUS-FNA were analyzed for KRAS exon 2 and exon 3 mutations, using four different techniques: Sanger sequencing, allele specific locked nucleic acid PCR, Next Generation sequencing (NGS 454 GS-Junior, Roche) and Idylla.

Results: The rate of mutant cases was as it follows: 26.2% Sanger sequencing, 35.7% allele specific locked nucleic acid PCR, 45.2% NGS and 40.5% Idylla. This latter proved to be more sensitive than Sanger sequencing and had a performance similar to NGS, as only two cases showing a very low mutant allele abundance by NGS (<1.5%) were scored as no mutation detected by Idylla.

Conclusions: This the first study validating KRAS mutation detection on clinical sample by Idylla. This proved to be a simple and potentially cost-effective technology which, may provide a rapid assessment of KRAS mutation status. The Idylla KRAS Mutation Test produces results in about 90 minutes with about 2 minutes of hands on time and the closed nature of the cartridge eliminates the risk of PCR contamination. Furthermore, given its simple workflow and quick turnaround time, the Idylla system can be used at nearly any facility.

Disclosure of Interest: None declared.

O-024

Clinical Utility of Next Generation Sequencing Using Touch Imprint Preparation Cytology

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Objectives: Elucidating the genetic alterations in tumor is needed for molecular targeting therapy. With advent of next generation sequencing (NGS) technology, genetic landscapes have been revealed in several types of tumors. In clinical setting, formalin-fixed, paraffin-embedded (FFPE) tissue is usually used for genetic analysis. However, FFPE DNA is occasionally not unusable for analysis due to the low amounts and fragmented DNA. Furthermore, it takes a couple of days to prepare FFPE sample. To provide early treatment for cancer patients, more simple and rapid method is required. This study was aim to examine the utility of cytology-derived DNA in genetic analysis to detect somatic mutations by targeted sequencing with multi-gene penal.

Materials and Methods: Touch imprint preparation cytology (TIC) and FFPE samples were obtained 9 lung carcinoma patients and 1 breast carcinoma patient during December 2014 to November 2015. To assess the integrity of DNA from FFPE and TIC, we performed quantitative real-time PCR using two primer pairs to estimate DNA fragmentation. First, we examined whether next generation sequence is capable using TIC-derived DNA using 9 lung carcinoma. Target sequencing was performed using custom designed panels in house: Lung Cancer Panel targeting 53 recurrently mutated genes. We next examined primary tumors (including triple negative (TN) and hormone receptor (HR) positive tumors) and metastatic tumor tissue for analyzing tumor heterogeneity in breast cancer patient using breast Cancer Panel targeting 53 genes.

Result and Conclusion: DNA quality of TIC was higher than that of FFPE. Sequence quality including the number of mapped reads, the proportion of mapped reads and uniformity was almost comparable between FFPE and TIC. In total 38 mutations were identified in lung primary tumor (19 mutations were from FFPE DNA and 19 were from TIC). Among these 17 mutations were consistent in both samples (89.5% concordance). Furthermore, TIC-derived DNAs showed different genetic profiles between TN and HR tumors. Clustering analysis showed genetic profiles of metastatic sites were significantly associated with that of HR tumor, but not of TN tumor, suggesting that analysis of tumor heterogeneity was capable using TIC-derived DNA. These results demonstrated TIC-derived DNA faithfully depicted the somatic mutations in tumor and applicable for NGS analysis for clinical diagnosis.

Disclosure of Interest: None declared.

O-025

Sensitive and Specific Real-Time PCR Methods for the Quantification of Telomerase Activity and hTERT Expression

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Objectives: Telomerase enzyme, containing the human telomerase reverse transcriptase (hTERT) and a small integral RNA component, is activated in almost all cancers. Thus, telomerase activity and hTERT might be universal and specific markers for diagnosing malignancy. The aim of the study was to develop sensitive methods to quantify telomerase activity and hTERT expression.

Materials and Methods: In this study, a reverse primer-linked probe (RPP), which combined the reverse primer and fluorescence labeled probe in one molecule, was used in a real-time TRAP assay for the quantification of telomerase activity. Molecular Beacon was used in real-time PCR assay for quantification of the hTERT expression.

Results: The sensitivity and linearity of the RPP-based real-time TRAP assay were evaluated by non-small cell lung cancer cell line A549. Using RPP-based real-time TRAP assay, telomerase activity could be detected in as few as one A549 cell. A linear relationship was also observed between telomerase activity and the logarithm of the number of cells ranging from 1 to 1000 cells. The correlation coefficient calculated from the linear regression model was up to 96.4%. In the quantification of hTERT, the molecular beacon probe could specifically examine the expression of hTERT.

Conclusion: We have developed highly specific and sensitive quantitative PCR protocols for determination of telomerase activity and hTERT. These methods could be used as adjunctive investigative tools in cytology assessment.

Disclosure of Interest: None declared.

O-026

DNA-Zwitterionic Liposomes – Me^{2+} Complexes: Role in Nuclear Pore Assembly and Gene Expression

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Objectives: The DNA forms complexes with lipids (DLC) both in vitro, and in vivo. The ternary complexes (TC): DNA-zwitterionic liposomes – Me^{2+} is only lately has received attention. Membrane vesicles forming the nuclear envelope with pores in anaphase cells are analogs of such liposomes. In accordance with our new nuclear pore model we proposed TC involvement in the nuclear pore assembly.

Materials and Methods: Calf thymus DNA, DNaseI was from (Sigma-Aldrich, USA), $MgCl_2 \cdot 6H_20$, $CaCl_2$ (Waco, Japan), DOPC and DOPE, Sphingomyelin (SM), PS, Cholesterol was from Avanti polar lipids, (USA); sucrose, glucose, NaCl, obtained from Wako (Japan).

Liposomes (LUV) from DOPC, DOPE and SM with addition another lipids were got by liposome extrusion in 0.01 M HEPES buffer, pH-7.5. Cryo-TEM experiments were carried out on a JEOL JEM-3100FFC electron microscope operated at 300-kV acceleration voltages with or without the Hilbert Differential Contrast phase plate (Lab. Prof. K. Nagayama, NIPS, Okazaki, Japan).

Result: The formation of TC accompanied by the aggregation and fusion of PC liposomes was shown by many biophysical techniques including EM (freeze-etching) and high resolution cryo-TEM technique. The double helix of DNA unwinds in the region of liposomes fusion. Membrane vesicles forming the nuclear pores in nuclei are analog of PC liposomes. In our last nuclear pore model TC arises in the chromatin areas with three-stranded hybrids: DNA – small nuclear RNA (snRNA) at their interactions with two small membrane vesicles (~70 nm in diameter). The thermo stability of DNA/snRNA triple helix is considerably lower than the same sequence of double-stranded DNA. That specifies preferential attachment of three-stranded hybrids to membrane vesicles. The triple helical hybrid unwinding during fusion of two membrane vesicles results in pre-pore formation: double-stranded DNA/snRNA hybrid and a single-stranded DNA (R-loop) located on the outer diameter of fused 'big vesicle'. This vesicle during interaction with double nuclear membrane can form channel between two membranes. During their fusion ssDNA and hybrid DNA/snRNA shifts to pore annulus center.

Conclusion: The ssDNA in pore annulus is the reason for the enhanced transcriptional activity of the genes neighboring nuclear pore. The number of pores in a nucleus specifies chromosome territory and number of chromosome loops. Thereby part snRNA or long non-coding RNA causing chromatin attachment to a membrane influences on gene expression, so on a cell differentiation. The ssDNA located along the outer diameter of 'big vesicles' serve as sites of transcription initiation and their aggregates can be considered as transcription factories.

Disclosure of Interest: None declared.

O-027

Splicing Factors and SWI/SNF Complex Regulate Mechanical Stress Induced Gene Alternative Splicing

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Objectives: Alternative splicing is found to be sensitive to mechanical stimuli through exome sequencing. However, the mechanism is poorly studied and our work is focused on mechano-regulation of alternative splicing. We want to know whether the alternative splicing of Cyclin D1 is regulated by mechanical stress and what kinds of factors may act as the regulator of mechano-induced alternative splicing.

Materials and Methods: In this study, we tested the alternative splicing of VEGF and Cyclin D1 in osteoblast cell lines and kera-

tinocyte cells loaded by a cyclic stretch. The cells were harvested at three hours and six hours after mechanical stress treatment. The expression of splicing factors and subunits of SWI/SNF complex were also detected in stretched cells. The recombinant plasmids of splicing factors and subunits of SWI/SNF complex were constructed and transiently transfected into keratinocyte cells respectively using Qiagen transfection regent. The alternative splicing of VEGF and Cyclin D1 were detected at forty eight hours post transfection.

Result: The results show that the expression of VEGF and two of its splice variants are significantly up-regulated, with the expression of cyclin D1 and its splice variant significantly decreased in cells after mechanical stretch. Three splicing factors and three SWI/SNF complex subunits are able to respond to mechanical stimuli.

Conclusion: The results demonstrate that several kinds of splicing factors and subunits of SWI/SNF complex are possibly responsible for the mechanical stress-induced up-regulation of VEGF, cyclin D1 and their splice variants.

Disclosure of Interest: None declared.

O-028

An Alternative Technical Method Suggestion for Diagnosis by Fine Needle Aspiration

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Objectives: Fine needle aspiration procedure is very important on patient diagnosis and management. Fine needle aspirations obtained from varied sites have been are admitted to our laboratory. Unfortunately, sometimes since the cellular materials may result thick smears, the evaluation may be difficult due to the lack of cellular details. immunohistochemical methods can be useful in overcoming this problem, but the unsufficient slide number and thick smearing may limit the quality of the evaluation. So that, here in, we report our attempt about an alternative method instead of diagnosed as 'nondiagnostic'.

Method: Firstly, lam was remowed from slide. Most thick area was scraped with lancet, wrapped in blotting paper and embedded parafine block. Routine tissue processing protocol was applied. Slides of 4 micron thickness were prepared, stained with hematxylene eosine and appropriated immunohistochemical antibodies.

Results:

Case 1: A patient presented with 2 cm sized lung mass was evaluated with these methods. Abortive glandular structures stained with CK7, Napsin, TTF1 were detected in cell block and diagnosed as adenocarcinoma. There was negative reaction with p63, CK5, Chromogranin and synaptophysine antibodies. The most important finality was implementation of EGFR mutation analysis by DNA sequencing and ALK-EML4 mutation analysis by FISH on this cell block.

Case 2: Same method was applied on FNA from lymp node. Specific immunohistochemical pannel was used and patient was diagnosed as lymphoma with B immunfenotype.

Conclusion: When we are in a helpless condition we should try to develop alternative solutions.

Disclosure of Interest: None declared.

O-029

Romanowsky Staining Using Liquid-Based Cytology

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Objectives: Liquid-base cytology (LBC) is widely used as standardized preparations for cytopathological examinations. To date, LBC technique are used for many kinds of specimens. However, Romanowsky staining has not yet been evaluated. Herein, we report a new technique for use of the Romanowsky-type (May-Grunwald-Giemsa, MGG) stain using a LBC technique (MGG-LBC).

Materials and Methods: KPL-1 human breast cancer cells at a density of 125,000 cells/20 ml were compared in conventional smear and MGG-LBC preparations. Cell size, nucleus/cytoplasm (N/C) ratio, and morphological findings were investigated. Each slide was scanned using a high-resolution digital scanner to prepare digital images. From the digital files, the 100 randomly selected cells within each smear to analyze cell size and N/C ratio. All values were expressed as mean ± standard error of the mean and analyzed using Mann-Whitney U tests. After analysis, we also investigated about some clinical samples (voided urine and pleural effusion).

Results: There was no significance of the cell sizes and N/C ratio of Papanicolaou- and MGG staining between conventional and LBC method clinical samples of pleural effusion revealed reactive mesothelial cells and ovarian adenocarcinoma cells, while voided urine samples revealed bacteria, crystals, eosinophils, normal urothelial cells, and low-grade urothelial carcinoma cells, sufficient for morphological diagnosis, in MGG-LBC preparations. Cellularity appeared lower with MGG-LBC compared with Papanicolaou stained smears using the LBC method.

Conclusions: We reported the utility of a novel preparation method for MGG-LBC. Romanowsky stains are used by many laboratories around the world and that experience with these preparations may be of value in educational and extradepartmental consultative work, even for those cytology professionals who do not rely on or use such in their own practices. Despite a lower cellularity than conventional techniques, MGG-LBC may provide useful supporting cytodiagnostic information.

Disclosure of Interest: None declared.

O-030

Usefulness of Ultraviolet-Microscopic Spectroscopy on Unstained Cells by Liquid-Based Cytology for Objective Differentiation between Non-Cancer and Cancer Cells

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Objective: The objectives of this study were to investigate the usefulness of Ultraviolet-microscopic spectroscopy (UV-MS) of unstained cells by liquid-based cytology (LBC) to objectively differentiate non-cancer from cancer cells.

Study Design: Cultured cell were used as samples: 100 non-cancer cells and 200 cancer cells (300 cells in total) were analyzed. The nuclear area measured by UV-MS was set at 166.4 μm^2 (12.9 x 12.9 μm). Transmittance of 260, 280, 300, 320 nm, and the 280 nm/260 nm, 300 nm/260 nm, and 320 nm/260 nm transmittance ratios were used for analysis. The regression equation to determine cancer cell probability (P(x)), discrimination predictive value, and odds ratio on binomial logistic regression analysis were investigated.

Results: The transmittance was lower in cancer (n = 200) than in non-cancer (n = 100) cells at all wavelengths, and the transmittances of all wavelengths of 260, 280, 300, 320 nm were significantly lower in cancer than in non-cancer cells ($p < 0.001$). All transmittance ratio was significantly higher in cancer cells than in non-cancer cells ($p < 0.001$). Two variances: of 280 nm/260 nm and the 320 nm/260 nm transmittance ratio, were finally extracted as effective items after applying forward and backward variance selection and investigation of multicollinearity. The binominal logistic regression equation to determine cancer cell probability (p(x)), was as follows (R^2: 0.48, likelihood ratio test: $p < 0.001$).

$p(x) = 1/(1+\exp(1.49+0.27x1-0.24x2))$,

$x1 = [280/260\%]$,

$x2 = [320/260\%]$

The result of the likelihood ratio test of the regression equation was $p < 0.001$, showing that this regression equation fitted. The accuracy of this linear discriminant function was 86.5%, and the sensitivity and specificity were 88.5 and 83.0%, respectively.

Conclusion: UV-MS on unstained cells by LBC yields an objective value to discriminate non-cancer from cancer cells. Since LBC is a sample processing procedure which will be increasingly used in the future, we are planning to further investigate its applicability to clinical specimens based on this study.

Disclosure of Interest: None declared.

Breast

O-031

Bright-Field HER2 Dual in situ Hybridization (DISH) Assay on Breast Cancer Cell Blocks: A Comparative Study with Tissue Sections

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Objectives: HER2 testing for samples from recurrent or metastatic disease is recommended by the 2014 update of the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines and cytological analysis can be applied to several types of metastatic lesions. However, the practical method to assess the HER2 testing of breast cancer cytology specimens have yet to be resolved. Therefore, we conducted the bright-field HER2 dual in situ hybridization (DISH) assay on cell blocks (CBs) prepared from breast cancer cell samples as a validation study before clinical use.

Materials and Methods: CBs were prepared from tumor cell samples collected from 54 surgically-excised breast tumors. The cells were fixed in 10% buffered formalin for 16 to 28 hours, and embedded in paraffin. The INFORM HER2/neu Dual ISH DNA Probe Cocktail was used for the DISH assay on the Ventana BenchMark ULTRA (Roche Diagnostics).

Results: Successful results were obtained in 51 of 54 CB specimens, and the results from the CB specimens were in agreement with those from the histological sections in 48 of the 51 cases (concordance rate, 94%; kappa, 0.846). The intraclass correlation coefficient (ICC) between the CB and histological specimens in the continuous HER2/CEP17 signal count ratio was 0.89 (95% CI, 0.81–0.93), and the Pearson's CC was 0.91 (95% CI, 0.85–0.94).

Conclusion: The HER2 DISH assay, utilizing 10% buffered formalin-fixed CB, would be a reliable and ideal method to assess the HER2 gene status of breast cancer cytological specimens.

Disclosure of Interest: None declared.

O-032

The Possibility of Determining Molecular Biological Subtypes of Breast Cancer by Cytologic Material

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The Purpose of Research: To study the possibility of determining the molecular biological subtypes of breast cancer by cytologic material.

Materials and Methods: Preoperative cytology Fine needle biopsies performed in 384 patients with breast cancer. In 252 pa-

tients with breast cancer compared preoperative and postoperative immunocytochemistry immunohistochemistry in determining the expression of estrogen receptors, progesterone, oncoprotein HER2/neu, protein proliferative activity of Ki-67 In 145 breast cancer patients with HER2-status undetermined defined possibility of FISH-studies on cytological material. Routine cytological preparations stained with azure-eosin dyes. Immunocytochemistry was performed using methods Ultra Vision, EnVision FLEX. Antibody was used firm 'Dako' estrogen receptor (ER), progesterone receptor (PR), oncoprotein HER2/neu, the proliferative activity of the protein Ki-67. Preparations for the immunohistochemical study was prepared by the method of liquid cytology by centrifugation (Cytospin 4, Thermo Scientiphic Shandon) and stained with the machine Autostainer 360, Thermo Scientiphic Shandon. FISH-reaction to use a set of firm 'Dako', including two-color probe to the gene HER2.

Results of the Study: Preoperative fine needle biopsy of mammary tumors cytology sensitivity was 97.9%, specificity 97.6%, accuracy 96.3%, 87.4% efficiency, bad material obtained in 8.6%. The percentage matches the results of immunocytochemistry and postoperative immunohistochemical study for estrogen and progesterone receptors was 88.6%, proliferative activity of the protein Ki-67–86% for HER2/neu – 93.2%. In the case of an indefinite immunohistochemical HER2-status (10%) of FISH-conducted study. The data obtained by amplification of the HER2 gene is fully correlated with the definition oncoprotein using immunocytochemistry. All data obtained by FISH-immunotsitohii and research allocated by subtypes according to molecular biological classification of breast cancer.

Conclusion: Fine-needle biopsy with cytology, immunocytochemistry and FISH-study – low-impact, safe, economical way to study the collection of material and cellular material from breast nodules, which allows in case a cancer to determine the molecular subtype of the tumor before beginning any treatment.

Disclosure of Interest: None declared.

O-033

Comparison of Two Methods for Evaluating the Ki-67 Labeling Index in Breast Cancer Based on Cytological Features

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Objective: Ki-67 labeling index (Ki-67 LI) reflects tumor's proliferative activity. Potential uses include prognosis and prediction of relative responsiveness or resistance to chemotherapy or endocrine therapy of breast cancer. However, it lacks standardized measurement methodologies. To evaluate Ki-67 LI, we compare the new method: PANASONIC PA (PA) with the conventional method.

Materials and Methods: Ki-67 LI was calculated by the immunohistochemical assessment of the proportion of cells which stained positive for the nuclear antigen Ki-67. We divided the Ki-67 LI results into three levels: high (30% or more), intermediate (between 20% and 30%), and low (less than 20%). To characterize cytological features at each of the three levels of Ki-67 LI, we reviewed 119 cytology samples. They included background of cytological findings: necrotic appearance, lymphocyte infiltration and mucus appearance. Regarding nuclear findings: high N/C ratio, nuclear margin irregularity, hyperchromasia and prominent nucleoli. Regarding nuclear size: minimum, maximum, average and standard deviation. Regarding other findings: multinuclear cells and intracytoplasmic lumen.

Results: 1. Regarding the Ki-67 LI results of PA: there were statistically significant differences among high, intermediate and low levels. This indicates that there is a correlation between Ki-67 LI of PA and cellular atypia.

2. The results of PA tend to be lower than the results obtained by the conventional method.

3. In one-half of the cases where the conventional method indicated high levels of Ki-67 LI, the PA method indicated low levels. Significant differences were observed in five aspects of cellular atypia: necrotic background, nuclear size (maximum, average and standard deviation) and prominent nucleoli.

Conclusion: The PA method is a promising new way to measure Ki-67LI. Based on the results, the PA method needs to be improved in order to consistently approximate the conventional method. We hope to present this study by using further improved PA data.

Disclosure of Interest: None declared.

O-034

Benign Phyllodes Tumor: Fine Needle Aspiration Cytology Findings

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Objectives: To describe the cytological findings of benign phyllodes tumor and to discuss the differentiation from fibroadenoma.

Materials and Methods: The study group consisted of 76 cases, aged between 12–36 years old. The sizes of tumors ranged between 3–12 cms. Clinically the cases were diagnosed as fibroadenomas. Later all cases were diagnosed histologically as benign phyllodes tumor. Eight smears were prepared from each case, four were air-dried and four were fixed in 90% ethanol and stained with Wright-Giemsa and Papanicolaou methods.

Results: The smears revealed hypercellularity, numerous large tree-like [Bonsai-tree] epithelial cell clusters, numerous bundles of spindle shaped cells, fragments of hyaline material, rare isolated epithelial and spindle shaped cells and rare bipolar naked nuclei. Interesting findings that were noticed in this study were: tree-like epithelial cell clusters, pop-corn like nuclei and intra nuclear inclusions. The cytological smears were diagnosed as benign phyllodes tumor versus cellular fibroadenoma. However histologically all cases were diagnosed as benign phyllodes tumor.

Conclusion: Sometimes it is very difficult to differentiate cellular fibroadenoma from benign phyllodes tumor. This study

shows that large tree-like epithelial cell clusters, pop-corn like nuclei and intranuclear inclusions can be helpful to differentiate benign phyllodes tumor from cellular fibroadenoma.

Disclosure of Interest: None declared.

O-035

Male Breast Disease: An Institutional Experience from South India

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Objective: Male breast lesions are uncommon as the male gender has less oestrogen when compared to females. However, in recent times the incidence is on the rise. There is not much literature from South India classifying the different benign, atypical and malignant conditions occurring in male breast diagnosed on fine needle aspiration cytology (FNAC).

Hence, the study is undertaken to classify male breast disease diagnosed on FNAC with the objective of understanding aetiologic factors.

Material and Methods: 120 cases studied over a period of 10 years i.e., January 2002 to December 2011, from the records of cytopathology, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh form the material for the study. The cytological specimens stained with routine Hematoxylin & Eosin, May Grunwald Giemsa and Pap techniques were studied.

Results: Age ranged from 14 to 80 years. Among 120 cases, 92 were benign, 11 malignant, 3 infectious and 14 inconclusive. Among benign, 61 were gynaecomastia, 23 fibroadenosis, 3 fibroadenoma, 3 lipoma, 1 duct papilloma and 1 fibrocystic disease. Malignant cases were all duct cell carcinoma. Suppurative inflammation seen in 3 patients is included under infectious category. Biopsy was taken as the gold standard and all FNAC diagnoses were confirmed by histopathology. Young males were affected by gynaecomastia, while elderly males were affected by carcinoma breast.

Conclusion: It is important to establish the correct diagnosis and aetiology. Thereby, affected patients are relieved from cosmetic disfigurement and infertility problems. Early treatment can be started in carcinoma breast.

Disclosure of Interest: None declared.

O-036

An Unusual Breast Tumor Identical to Solid Papillary Carcinoma

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I encountered a breast tumor identical to solid papillary carcinoma. The histological and cytological features with immunohistochemical characteristics of this tumor are described.

Case: A 76-year old woman underwent fine needle aspiration (FNA) of a 1.9 cm palpable right breast tumor. The smears showed hypercellularity with many cohesive papilalike clusters of epithelial cells and many isolated cells, in a clean background. The tumor cells exhibited round to oval nuclei with mild nuclear pleomorphism. Their chromatins were finely granular. The isolated cells were spindle in shape with obvious fibrils. Their chromatins likewise were finely granular. The patient received core needle biopsy which showed multiple nodules, each representing a duct filled by proliferating neoplastic cells. The cells grow in a solid pattern with spindle cell predominating. Mitotic figures were uncommon. Immunohistochemical staining using anti smooth muscle antibody showed absence of myoepithleial cells around each nodule. Using anti-pancytokeratin, the neoplastic cells were all strongly positive. The tumor was interpreted as spindle cell carcinoma. Subsequently, the patient underwent a partial mastectomy. A well circumscribed grayish white tumor was found, measuring 1.9 cm in its greatest dimension. The histological examination displayed a similar morphology as that of biopsy specimen. More immunohistochemical stainings were performed, the results were as follows; ER2+, diffuse; PR 3+, diffuse; Her2/neu, 3+, diffuse; Ki67:5%, CK 5/6, negatve; vimentin, negative; Synaptophysin, strongly positive; chromogranin, focally positive. Special stain for mucin was negative.

Conclusion: Given the biphasic cell population in the FNA smears, the differential diagnosis ranges from benign to malignant lesions including metaplastic carcinoma, spindle cell ductal carcinoma in situ, phyllodes tumor, florid ductal hyperplasia. Low nuclear/ctoplasmic ratio and mild nuclear pleomorphism with clean background ruled out spindle cell ductal carcinoma in situ. That neoplastic cells were positive for cytokeratin and negative for vimentin ruled out metaplastic carcinoma. Histologically, the tumor appeared as multiple nodules, identical to solid papillary carcinoma (SPC) with neuroendocrine differentiation. However, FNA smears and histological sections failed to demonstrate plasmcytoid cells or mucinous material. Ki 67 was only 5%. In conclusion, I described the cytologic and histologic features with immunohistochemical characteristics of an unusual spindle cell breast tumor which does not fall within the general category of metaplastic carcinoma. The tumor has neuroendocrine differentiation, and its growing pattern is similar to SPC. The tumor may represent a variant of SPC.

Disclosure of Interest: None declared.

O-037

Eight Cases of Metaplastic Breast Carcinoma, Cyto-Histo-Gross Pathological and Clinical Study

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Introduction: Metaplastic breast carcinoma (MBC) is a rare heterogeneous group of primary breast malignancies accounting for less than 1% of all invasive mammary carcinomas. They are characterized by the co-existence of adenocarcinoma with spindle tumor cells, squamous tumor cells, or non-epithelial stromal cellular elements. We studied 8 cases of MBC.

Materials and Methods: We found 8 cases of MBC in archival materials of our pathological laboratory (2001–2015). The protocols, histological and cytological preparations of the cases were analyzed and re-assessed.

Results:

1. Clinical Findings: The age of the cases ranges from 39 to 93 of years, and the average ± standard deviation was 64 ± 19. All are Japanese females.

2. Gross and Histopathological Features: The primary tumors located in A-area in 2 cases, in B-area in 2 cases, in C-area in 1 case, and in E-area in 2 case. In one case the tumor was so large that the primary location was indeterminate. The sizes of the primary tumors ranged from 0.5 cm to 25.0 cm in largest dimensions. The average ± standard deviation were 5.26 cm ± 7.56 cm. Histopathologically all the 8 cases contained elements of squamous cell carcinoma (SCC). The tumor of 2 cases consisted of all most pure SCC. In other 4 cases, they consisted of combination of SCC and spindle cell carcinoma, and 2 of them included multinuclear giant cells in addition. In 5 cases, they consisted of adenosquamous carcinoma. Two cases of them were -low grade adenosquamous carcinoma-, one of the 5 classifications proposed by the WHO working group.

3. Cytological features: In 4 cases, cytological findings were available. The cases comprised of 2 cases of adenosquamous carcinoma, 1 case of low grade adenosquamous cell carcinoma with spindle cell carcinoma, and SCC associated with spindle cell carcinoma and multinuclear giant cells. Cells of squamous cell carcinoma were detected in all the 4 cases. Cells of spindle cell carcinoma were not detected in primary diagnosis, but, they were recognized in re-assessment after histopathological evaluations. Multinuclear giant cell, although it was present only in one case, was detected in primary cytological diagnosis.

Discussion and Conclusions: MBC is a disease of middle aged or old-aged females. It has no apparent predilection regarding location in the breast and the size of primary focus. Most prevalent metaplastic element in MBC is SCC, followed by spindle cell carcinoma. Most prominent cytological feature for MBC is the cells from SCC. Since cells of spindle cell carcinoma are prone to be overlooked in primary cytological diagnosis, spindle cells should be searched carefully in the cytological preparations of the breast tumors especially when we find cells of SCC in them.

Disclosure of Interest: None declared.

Miscellaneous

O-038

FNA of Lesions Yielding Keratinized Squamous Epithelial Cells: Cytomorphologic Overlap, and Significance of Optimal Cytopreparation

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Objectives: Fine needle aspiration (FNA) biopsy of lesions yielding keratinized squamous epithelial cells (KSEC) may pose diagnostic challenges. This problem may be accentuated when cytomorphologic or diagnostic criteria may be absent or obscure. KSEC in FNA cases may represent squamous cell carcinoma (SCC); however benign lesions may also yield similar cells and cytomorphologic features. This study reviews FNA cases yielding KSEC to explore the cytomorphologic overlap between cases of SCC and those reflecting benign lesions. This study also raises awareness of potential diagnostic limitations discussing technical approaches that may facilitate reporting in such cases.

Materials and Methods: Reviewed cytopreparations [i.e. smears (Papanicolaou and Diff-Quik stained), cell block sections (H&E stained)] from FNA procedures sampling lesions for which the presence of KSEC was reported. Six cases were reviewed comprising: (a) keratinizing SCC (trichilemmal cyst mass); (b) epidermal inclusion cyst; (c) thyroglossal cyst; (d) branchial cleft cyst; and (e) anaplastic carcinoma with squamous differentiation (thyroid nodule). Representative photomicrographs were organized to emphasize cytomorphology.

Results: Cytomorphologic overlap included the following squamous cell features: mature and immature cells; anucleated and nucleated cells; hyperchromatic and pyknotic nuclei; variable degrees of keratinization; dense eosinophilic and orangeophilic cytoplasm. Backgrounds included: keratin and fragmented cytoplasmic deposits; necrotic and proteinaceous debris; and infrequently, cell whirling with spindle shapes. Cytodiagnoses of KSEC from lesions sampled through FNA should take into account: lesion physical nature; anatomic location; adequacy of FNA and cytopreparatory practices; and consideration of recognized diagnostic pitfalls. Prominent nucleoli were rare in KSEC from the benign lesions; hence may be a reliable marker for SCC.

Conclusion: Based on the identification of KSEC, SCC is a foremost consideration to be ruled out. Optimal FNA methodology is key for adequate lesion sampling. Cytopreparation with immediate alcoholic fixation, eliminating air-drying, is crucial for proper Papanicolaou staining results ensuring cellular transparency and clear visualization of nuclear and cytoplasmic elements. Air-dried preparations may pose limitations as Diff-Quik stains keratin weakly, and hyperchromatic nuclear detail may not be adequately evident. Cell blocks are advantageous as they may reveal cellular architectural morphology in larger groups that may otherwise be difficult to assess due to thickness or obscuration. Cell block sec-

tions proved highly useful in the reporting of the cases reviewed for this study. Careful, multi-plane microscopic inspection cannot be over-emphasized.

Disclosure of Interest: None declared.

O-039

The Diagnostic Value of Fine Needle Aspiration Cytology by Cell Blocks of Paraffin Section for Superficial Masses

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Objective: To investigate the diagnostic value of fine needle aspiration cytology (FNAC) smear and paraffin sections of cell blocks in the qualitative diagnosis and tumor classification for superficial masses.

Methods: Under 6–10 ml negative pressure, using a common fine disposable 10 ml syringe with a needle only 0.6–0.7 mm in diameter performed cytology examination for 216 cases of superficial masses. The cytology examination included cytological smear, paraffin sections of cell blocks, and special dyeing or immunohistochemistry or molecular pathology detection chose by cell morphology and diagnostic need. Then the results of cytology examination and postoperative pathological histology examination were compared and analyzed.

Results: Accurate rate was 97.6% (80/82) in 82 cases of breast lesion, 96%(72/75) in 75 cases of cervical lesion, 95.5%(21/22) in 22 cases of thyroid nodule, 100%(5/5) in 5 cases of head and face lesion, 88.9%(8/9) in 9 cases of limbs lesion, 100% (7/7) in 7 cases of chest and abdominal lesion, 100% (11/11) in 11 cases of axillary lesion, 80%(4/5) in 5 cases of inguinal lesion. The total accurate rate was 96.3% (208/216). And the accurate rate of metastatic tumor classification was 98.2% (56/57).

Conclusions: The diagnostic accurate rate of the combination of cytological smear and paraffin section of cell block is high for superficial masses, but there is not obvious cytology characteristics in tuberculosis and tumor lesions only by cytological smear, especially in granulomatous inflammation and poor differentiation lesions. The method that special dyeing or immunohistochemistry or molecular pathology detection is performed for the paraffin sections of cell blocks can improve the accuracy of diagnosis and classification and provide reliable pathology diagnosis basis and theoretical directions for clinical targeted therapy and precision medicine.

Disclosure of Interest: None declared.

O-040

Correlation of Eosinophilic Structures with Detection of Acid-Fast Bacilli in Fine Needle Aspiration Smears from Tuberculous Lymph Nodes: Is Eosinophilic Structure the Missing Link in Spectrum of Tuberculous Lesion?

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Objectives: Acid-fast bacilli (AFB) is not seen in all necrotic tuberculous lesions. If the subset of tuberculous lesions which yield positive result for AFB can be identified, it would save on time and manpower besides optimizing use of resources. A prospective study was undertaken to assess if presence of eosinophilic structures (ESs) in necrotic tuberculous lesions correlated with the presence of AFB.

Materials and Methods: Patients referred for fine needle aspiration cytology for evaluation of lymphadenopathy between July 2012 and June 2013 were analyzed. The Hematoxylin and Eosin and May-Grünwald-Giemsa stained slides were screened for epithelioid cell granuloma, ES and necrosis and Ziehl Neelsen stained smears for AFB.

Result: One hundred and eight tuberculous lymph nodes yielded necrotic material on aspiration. Four cytologic pictures were seen: (a) ES+ AFB+ in 58.33%, (b) ES+ AFB– in 20.37%, (c) ES– AFB+ in 9.26% (d) ES– AFB– in 12.04% cases. Overall AFB was found in 67.59% cases, out of which 58.33% correlated with the presence of ES while 9.26% were seen in smears without ES.

Conclusion: Presence of ES should be included in the morphological description of tuberculous lesions. In the absence of granulomas, they indicate tuberculous nature of the lesion. Presence of ES mandates a search for AFB as probability of finding AFB is high in such lesions. Significance of ES lies in their presence and not in their absence. Eosinophilic structures appear to be the missing link in the spectrum of tuberculous lesion.

Disclosure of Interest: None declared.

O-041

Identification of A11 for Melanoma Treatment and Skin-Whitening

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Introduction/Objectives: Melanocytes are the major pigment producing cells that determine the skin tones in humans and the unique color patterns in animals. Chemical intervention to obtain desired skin tones or treat pigment abnormalities has been practiced worldwide in the cosmetic industry. However, the mecha-

nisms and toxicity are still not fully understood for many of the skin-whitening compounds. We previously identified a family of phenolic compounds with the lead compound named A11 that exhibit pigment reduction ability. In this report, we describe the characterization of A11 and several known skin-lightening compounds.

Materials and Methods: We employed both in vitro, the mouse melanoma cell line B16-F10, and in vivo, zebrafish embryos, melanogenesis models to study the cellular and molecular mechanisms of A11 comparing with compounds currently in cosmetic products.

Results: Like arbutin, A11 showed mild melanin reduction in melanoma cells. However, A11, but not arubtin, significantly inhibited melanoma cell proliferation. Unlike other compounds, A11 is not an inhibitor of tyrosinase, the key enzyme for melanogenesis. The effect of A11 on melanoma cell lines is through inhibiting phosphorylation of Akt but activating MAPK. On zebrafish embryos, A11 and arbutin both showed strong pigment reduction during and post melanogenesis. Using a melanoma transgenic zebrafish model, we found that A11 can effectively inhibit the melanoma formation. Furthermore, pigment recovery occurred within 24 hours after withdrawal for most known skin-whitening compounds but much slower for A11.

Conclusion: Our results suggest A11 to be a potential compound for both melanoma chemotherapy and skin-whitening.

Disclosure of Interest: None declared.

O-042

Fine Needle Aspiration Cytology of Parasitic Cysts

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Objective: To analyze the morphological features of parasitic cysts and study the utility of fine needle aspiration in their diagnosis.

Material and Method: All fine needle aspirates diagnosed as cysticercosis or hydatid cysts over a 15 year period from *January* 2000 to December 2015 were re-evaluated for cytomorphological features.

Results: The study included 128 cases of cysticercosis, presenting with palpable nodules at varied anatomic sites. These included neck region (38 cases, 29.67%), trunk (36 cases, 28.13%), extremities (34 cases, 26.56%). Uncommon site included cheek (6 cases, 4.69%), breast (3 cases, 3%), axilla (5 cases, 3.9%), mandible (2 cases, 1.56%), tongue (1 case, 0.78%), parotid gland (1 case, 0.78%), sacroiliac joint (1 case, 0.78%), and liver (1 case, 0.78%). Aspirate yielded clear fluid in 33 cases (25.78%), pus in 15 cases (11.71%) and blood mixed aspirates in the remainder. Diagnosis of cysticercosis was made on identification of bladder wall in 121 cases (94.53%). Aspirates showed well preserved parasites in most of the cases with degenerated parasites in a few accompanied by calcospherules and giant cells. Histiocytic collections were identified in over half the cases. Hooklets were seen in 7 cases (5.47%) which showed acute inflammatory exudate and necrotic material. Fourteen cases of hydatid disease were seen. They showed hooklets and laminated membrane in 12 cases (85.71%) while 2 cases (14.29%) showed complete scolices.

Conclusion: Cysticercosis and hydatid cysts are endemic to India and aspiration cytology features play an important role in diagnosis. Identification of calcospherules and giant cells in necrotic cysticercosis should prompt a search for hooklets of cysticercosis. Hydatid cysts are diagnosed on radiology however in suspected cases when aspirated, should be diagnosed on fine needle aspirate using charactersistic morphological features.

Disclosure of Interest: None declared.

O-043

Cytotechnology Educational Program in Kitasato University

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The training and educational system of the Cytotechnologist (CT) varies by country. We aim to summarize the educational system in Japan along with our history as the pioneer University which runs University-based CT program. In Japan currently there are three ways to be eligible for taking examination to be a CT; 1) Medical Technologist (MT) who practiced more than one year in a cytology laboratory as a trainee, 2) MT who studied and graduated a special CT school or 3) to-be-MT undergraduate who undertook accredited Cytotechnology educational program in University as a part of MT program.

Kitasato University was founded in 1962 as the first school for the professional education of specialists in the fields of Hygienic Sciences and Medical Technology and Kitasato University now is recognized as a leading university promoting education specialized in life sciences. In 1994, the School of Hygienic Science was reformed to the School of Allied Health Sciences and the Bachelor's Degree Educational Program of CT was also established as the part of the MT program for the first time in Japan. In our program students will obtain two certificates, MT and CT, upon the success on their examinations. As well as learning Cytotechnology students also learn basic molecular pathology research techniques and write a thesis for their Bachelor's Degree. This year (2016), we celebrate our 20th anniversary. So far more than 200 students have gone through our educational program and the graduates are now working in worldwide.

We have been running Student/Faculty Exchange Program with School of Health Professions in Philadelphia, Thomas Jeffer-

son University, U.S.A. since 1997. The exchange students had the privilege to join the regular classes and learn Cytotechnology and Medical Technology for three weeks. This exchange program has contributed to enhance students' motivation for learning Cytotechnology as well as improving the knowledge in cytology.

As a leading University for a healthcare and life science we have a duty to contribute to the nation's health care through not only maintaining the level of Cytotechnology but also keep improving our skills and knowledge. HPV-DNA test and HPV vaccination may also change in the discipline of cytology. However, morphologic diagnosis skills will still be required for the future. We should keep evaluating our healthcare system and technologies over a time, and adapting and developing our CT educational program as necessary in the future.

Disclosure of Interest: None declared.

O-044

An Audit of a Pathologist Performed US-Guided FNAC Out Patient Clinic; The AHUS Institutional Experience

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Objective: Review our institutional experience with pathologist-performed US-guided examination and FNAC of superficial lesions.

Material and Methods: A total of 174 FNAC cases were reviewed. They had been performed during 2 ½ yrs. The patients were referred from general practioners outside the hospital and from the various departments in the hospital. Only superficial lesions were aspirated. Patients were seen on one morning session per week. US examination and FNAC was done by two dedicated cytopathologists.

Results: There were 174 cases including 63 lymph nodes (36.2%), 20 head and neck NOS (11.5%), 17 salivary glands (9.7%), 39 thyroid (22.4%), 18 soft tissue NOS (except head and neck) (10.3%) and 6 breast lesion. In 11 referred cases no palpable and/or ultrasound visualized lesion was found and FNAC was not done (6%). Malignancy was diagnosed in 17/174 lesions (9.8%). These comprised 5 lymph node metastases (2 malignant melanomas and three carcinomas), one acinic cell carcinoma in the parotid gland, 3 papillary thyroid carcinomas, two breast carcinomas (IDC), one subcutaneous metastasis from MM, three malignant lymphomas, one epitheloid hemangioendothelioma and one basal cell carcinoma of the skin. In 7 cases no diagnostic material could be obtained (4%).

Conclusions: 80% of the lesions were in the head and neck region (57.5%) plus thyroid. US examination is a valuable tool to visualize the lesions, secure material from relevant regions of the lesions and to identify cases not representing any focal lesion or tumour. The rate of non-diagnostic material is low.

Disclosure of Interest: None declared.

O-045

Quality Assessment of Cytological Diagnosis of Lung Cancer Using Hospital Cancer Registry

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Objective: For quality assessment of cytological diagnosis, its results were referred to those of histological Diagnosis using the data recorded in anatomical pathology information system (APIS). However, the technique does not work well on the organs in which a relatively high proportion of cancers were not proved histologically, such as lung. Hospital cancer registry (HCR) records all cancer cases diagnosed and/or treated in a hospital including those not pathologically proved. We reviewed the advantages of HCR as another source of comparison of the cytological Diagnosis.

Materials and Methods: The results of cytological Diagnosis of respiratory specimens including bronchial lavage, brushing and sputum were extracted from APIS of our hospital to an Excel file. The cytological Diagnosis of malignancy was recorded as 'ClassV'. Data were processed with some basic functions such as 'vlookup', 'if', and 'countifs'. Repeated diagnoses in a patient were summarized to the highest category and the results were linked to the HCR (2007–12) using a patient's ID.

Results: The recorded cytological diagnoses were summarized to 1000 cases, (230 ClassV, 742 negative, and 28 *indeterminate* cases; 343 lung cancers in HCR). Among the ClassV cases, 11 had no lung cancer record on HCR. Checking through only 11 medical records proved that there were no false positive. The 24 of 28 *indeterminate* cases had lung cancers, which might be controversial. The 742 negative cases had 87 lung cancer registries. Of the 39 lung cancers in HCR without histological Diagnosis records in APIS, 20 were ClassV cases.

Conclusion: The comparison of cytological diagnosis recorded on HCR was easy. We could find out the candidate for false positive and false negative using HCR. Although APIS remains a very important source for comparison, the assessment using both HCR and APIS is recommended in the institutions having reliable HCR, especially on the cancers of which histological examination is often difficult, such as pancreas cancer, biliary tract cancer and lung cancer.

Disclosure of Interest: None declared.

O-046

Total Quality Management in ISO15189; 2012 Certified Cytopathology Laboratory: Five Year Experience in Resource Limited Setting

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Introduction: Quality control is backbone of any laboratory but this concept is relatively difficult to implement in cytopathology laboratory due to non-uniform descriptive reports and subjec-

tive variability. Total quality management includes pre-analytical, analytical and post-analytical phases with external quality assessment to minimize or eliminate errors resulting in accurate cytopathology reports. Present study describes components followed for quality assessment of analytical phases with various measures adopted for quality management of cytopathology laboratory in resource limited setting.

Material and Methods: Study evaluated components of pre-analytical, analytical and post-analytical phases including documents, inter-laboratory comparison, system procedure analysis and external quality assessment which were followed over period of five years in ISO certified cytopathology laboratory in medical institute.

Results: Total 18564 samples were subjected to cytopathological examination including FNAC, fluid cytology, gynecological smears and exfoliative cytology over the study period. Pre-analytical aspect included properly filled request form with unique identification number, stain validation record, standard operating procedure, fixative/chemical change record and repeat cytology record. 297/18564 cases (1.6%) showed pre-analytical error which was most commonly (152 cases) due to inadequate sampling in which on site evaluation was not done or due to aspirator inexperience. Analytical aspect included reporting system which was either internationally accepted or according to institutional protocol with random blinded case review by the same or different person along with cyto-histopathological correlation. Major discrepancy in diagnosis was observed in 28 cases (0.15%) which were subjected to external experts. Post-analytical aspects included transcription of final report, report turnaround time record, archiving of smears, forms along with proper disposal of specimen after suitable retention time. 232/18564 cases (1.2%) showed post-analytical error with maximum frequency due to delayed turnaround time or typographical error. Participation in External Quality Assessment Program with accredited institutes were done yearly with satisfactory results.

Conclusion: Total quality management should be practiced in every cytopathology lab to ensure accurate and precise reporting with maintenance of standard protocols. Vigilant review of errors followed by sincere effort to minimize them may be highly effective for adequate quality control even in limited resources. In addition, participation in external quality assessment program may further add to quality improvement of cytolopathology laboratory.

Disclosure of Interest: None declared.

O-047

Sensory Cutaneous Nerve Fine Needle Aspiration in Hansens Disease

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Objectives: Leprosy affects peripheral nerves. As Mycobacterium leprae has unique tropism for Schwann cells, thickened sensory cutaneous nerves provide an easy target for the detection of lepra bacilli and other changes associated with the disease. This retrospective study was undertaken to analyze the feasibility, efficacy and role of fine needle aspiration cytology of sensory cutaneous nerves in Hansen's disease.

Materials and Methods: The data of patients with sensory cutaneous nerve involvement were retrieved from our record for the period January 2006 to December 2014. The hematoxylin and eosin and May-Grünwald-Giemsa stained slides were screened for Schwann cells, granuloma, and necrosis. Modified Ziehl-Neelsen stained smears were searched for lepra bacilli and globi. Morphological index was calculated in multibacillary lesions.

Result: Twenty nine sensory cutaneous nerves were aspirated in 23 patients. While 15 cases showed skin and nerve involvement, 8 cases showed only nerve involvement. Terminal cutaneous branch of the radial nerve was most often aspirated. No motor loss was observed after aspiration. Five cytologic pictures were seen – Epithelioid cell granuloma only in 6 cases, epithelioid cell granuloma with necrosis in 1 case, epithelioid cell granuloma with lepra bacilli in 3 cases, necrosis with lepra bacilli in 1 case, and only lepra bacilli in 12 cases. Morphological index ranged from 20% to 80%.

Conclusion: Sensory cutaneous nerve fine needle aspiration is a feasible, viable, effective and safe procedure. It adds to diagnostic FNA yield in patients with concomitant skin involvement and offers a way to evaluate patients with only nerve involvement. Calculation of morphological index allows prognostication and may have a role in assessing response to therapy and/or relapse.

Disclosure of Interest: None declared.

O-048

Embryonal Carcinoma of the Testis; Cytodiagnosis, A Case Report

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Objectives: The purpose of this study was evaluation of intraoperative scraping cytological method in diagnosis of a testis tumor and define diagnostic criteriae in order to apply in all testicular specimens.

Case: A 32 y/o man presented to the clinic with testicular enlargement. The surgical specimen consisted of testis and spermatic cord. The testis was 5x4.5x3.5 cm. with smooth surface. There was a central tumor in cut surface measuring 4x3x2.5 cm. pink and yellow in color, firm on cutting and with irregular borders. The epididymis and distal portion of the cord were firm and tumoral. Scraping smears of the tumor were prepared for intraoperative cytological diagnosis and stained by rapid H&E method.

Results: The smears were cellular and composed of epithelial-type cells arranged mostly in sheets and clusters and fewer single cells. Tumoral cells had large vesicular and pleomorphic nuclei with irregular borders and reticular chromatin pattern. The nucleoli were large, highly atypical and irregular. Mitotic activity was seen. The cytoplasm was pink and there was evidence of necrosis and hemorrhage. Cytological report was a highly malignant germ cell tumor suggestive for embryonal carcinoma. Histological ex-

amination revealed solid clusters and irregular spaces lined by several layers of columnar to polyhedral cells and papillary formations were encountered. Histological findings were those of Embryonal carcinoma.

Conclusion: Cytology of embryonal carcinoma of the male gonad has rarely been described in the literature. FNA of the testes is not practically done as it is painfull and not tolerated by the patient. Intraoperative cytological diagnosis of testis tumors by scraping method is not difficult and is of great value in pure seminoma and embryonal carcinoma and helps urologist for appropriate management of the patient during operation. More study is necessary in this field to find cytological criteriae in other testicular neoplasms such as teratoma, mixed germ cell tumors and non-germ cell category.

Disclosure of Interest: None declared.

Gastrointestinal, Liver and Pancreas

O-049

Endoscopic Ultrasound-Guided Fine-Needle Aspiration Biopsy for the Diagnosis of Gastrointestinal Stromal Tumors in the Stomach

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Introduction: Gastrointestinal stromal tumor (GIST) is a primary mesenchymal neoplasm that recently was defined by its ultrastructural and immunohistochemical similarity to the interstitial cells of Cajal. Diagnostic importance of the cytopathologic evaluation of these tumors is increase in since endoscopic ultrasound-guided fine-needle aspiration biopsy was come in to use.

Case: Fifty years old patient was admitted to gastroenterology clinic with abdominal distention and pain. Seven cm sized submucosal mass extending to the retroperitoneal area was detected in stomach curvature minor omental side with abdominal CT. Upper gastrointestinal endoscopy was planned for diagnosis. There was no mucosal lesion seen in endoscopy. Endoscopic ultrasound-guided fine-needle aspiration biopsy was performed for the diagnosis of lesion. Smear prepares stained with PAP and liquid base cytology material were evaluated in our department. Three dimensional groups or individual cells with spindle shaped, large and pleomorphic nuclei were seen in proteinous background. Cytological samples were typically showed irregularly outlined clusters of spindle cells that were spread easily without crush artifact. The cells had wispy cytoplasm with long, delicate, filamentous extensions. The patient was diagnosed as 'a mesenchymal lesion; gastrointestinal stromal tumor with high risk or leiomyosarcoma should be considered for differential diagnosis'. High SUVmax value was seen in PET/CT (SUVmax: 10.5). Tumor excision and partial stomach resection were performed. Histopathological evaluation was compatible with high risk gastrointestinal stromal tumor. High cellularity, pleomorphism, high mitosis count (>5/10HPF), high Ki 67 proliferation index (>%10), CD117, SMA, DOG 1 positivity were used for support to the diagnosis.

Conclusion: GISTs show a wide spectrum of cytological features and the presence of mitosis, necrosis and nuclear pleomorphism can help in prediction of malignant behavior. Cytological features of GIST should be evaluate carefully.

Disclosure of Interest: None declared.

O-050

Cytologic Characteristics of Circulating Epithelioid Cells in Pancreatic Disease

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Objectives: Circulating epitheloid cells (CECs), also known as circulating tumor, cancer, epithelial, and non-hematologic cells, may be a prognostic factor in various solid malignancies. Methods for isolating CECs from blood include size-based filtration and immunological-based capture methods. We analyzed the cytomorphologic characteristics of CECs isolated by size in a cohort of patients with pancreatic disease.

Materials and Methods: The study cohort consisted of 144 patients with pancreatic disease presenting for surgical evaluation, as well as 9 healthy controls. Blood samples were obtained before treatment and were processed with the [ScreenCell] size-based filtration device. Evaluable CECs were detected in 86 patients and were analyzed in a blinded fashion for cytomorphological characteristics including cellularity, nucleoli, nuclear size/irregularity/variability/hyperchromasia, and nuclear-cytoplasmic ratio. Statistical differences between variables were analyzed via Fisher's exact probability test.

Results: No CECs were identified in controls. Of the 86 patients with CECs, 63 had malignant disease including 47 pancreatic ductal adenocarinomas (PDAC), 6 neuroendocrine tumors (NET), 4 cholangiocarcinomas, 4 duodenal adenocarcinomas, and 2 solid-pseudopapillary neoplasms. 23 patients had non-malignant disease, including 12 intraductal papillary mucinous neoplasms (IPMN), 7 chronic pancreatitis, 2 serous cystadenomas, 1 mucinous cystic neoplasm, and 1 duodenal adenoma. There were no statistical differences in any of cytologic criteria between groups divided by non-malignant versus malignant disease ($p > 0.05$). There were also no differences in CEC characteristics between specimens from patients with PDACs and NETs ($p > 0.05$).

Conclusions: CECs were seen in patients with malignant and non-malignant pancreatic disease, but not in healthy controls. Neither cytologic characteristics nor analysis by experienced pathologists were able to elucidate any morphologic differences between cells from different pancreatic diseases. This implies that numerous conditions may be associated with CECs in the circulation and that care must be taken not to over-interpret cells identi-

fied by cytomorphology as indicative of circulating tumor cells of pancreatic cancer. Additional studies are required to determine the origin and clinical significance of these cells.

Disclosure of Interest: None declared.

O-051

Diagnosis of Pancreatic Solid Pseudo-Papillary Neoplasm by Fine Needle Aspiration Cytology (FNAC): Unusual Presentation

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Objective: The cytologic diagnosis of solid pseudo-papillary neoplasm (SPN) in old males is frequently hampered by its rarity and resemblance to various tumors. This work demonstrates the role of FNA in diagnosing SPN with an unusual presentation of age and gender.

Material and Methods: A 56 Y-O male presented with pancreatic tail mass for sonar guided FNAC. US-guided FNA was performed using 21 gauge needle. Two passes were made by transabdominal approach. Smears were prepared with immediate 95% ethanol fixation for Papanicolaou stain and additional smears were air-dried for on site rapid evaluation (ROSE) using Diff-Quick stain. Additional material was secured for cell block preparation for immunocytochemistry. The cytologic diagnosis was followed by distal pancreatectomy.

Results: Cytologic examination revealed groups and sheets of monomorphic cells loosely aggregated in single or multiple layers around arborizing vessels which are expanded by myxoid and hyaline material. These cells had delicate pale cytoplasm with indistinct cell borders. Nuclei were uniform round to oval with fine, evenly distributed chromatin and occasional grooves. Tumor cells showed strong immunostaings for B-catenin and Progesterone receptor with focal positive reaction to synaptophysin and CK. Such result confirmed the diagnosis of SPN tumor based solely on FNAC which was confirmed by subsequent distal pancreatectomy and histology diagnosis.

Conclusion: Cytologic diagnosis of solid pseudopapillary tumor of the pancreas can be reliable with the help of immunocytochemistry on cell block preparation not only in clinically typical cases but even in unusual presentations such as in older and male patients.

Disclosure of Interest: None declared.

O-052

Percutaneous Ultrasound-Guided Fine Needle Aspiration in the Evaluation of Pancreatic Lesions: an Eleven Year Retrospective Study in a Tertiary Institution

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Objectives: The purpose of this study was to evaluate the diagnostic accuracy of ultrasound (US) guided FNA of pancreatic lesions with a comparison to concomitant or core biopsies and/or subsequent surgical resections.

Materials and Methods: The data was collected from a retrospective review of pathology reports of FNA and biopsy samples of pancreatic lesions at the University Health Network during the period from 2003 to 2014. Cytology diagnoses were revised according to 2014 standardized terminology by Papanicolaou Society and correlated with biopsy diagnoses.

Results: A total of 168 samples from 164 patients (84 F, 80 M) were included. Median age was 62 years (range 58–84). Adequate FNA sample for the diagnosis was obtained in 136 cases (81%). Cytologic diagnoses include 105 positive/malignant, 4 suspicious, 7 neoplastic, and negative in 20 cases. Malignant diagnoses include 93 adenocarcinomas, 1 adenosquamous carcinoma, 1 small cell carcinoma, 1 renal cell carcinoma, 2 melanomas, 2 lymphomas (1 follicular lymphoma and 1 diffuse large B cell lymphoma), 1 plasmacytoma, and 4 carcinomas, NOS. The concurrent core biopsy was performed in 90 cases (53.6%). Discrepancy between FNA and biopsy results was observed in 17 cases (18.9%). The most common reason for the discrepancy was non-diagnostic FNA sample observed in 10 cases. Sixty cases (35.7%) were diagnosed as malignant by FNA alone. Subsequent surgical resections confirmed malignancy in four of these cases.

Conclusions: Percutaneous US guided FNA alone can be a reliable tool for the diagnosis of a wide range of malignant lesions of the pancreas including primary, secondary and hematologic malignancies.

Disclosure of Interest: None declared.

O-053

The Role of Fine Needle Aspiration (FNA) in the Detection of Common and Uncommon Lesions of the Pancreas: A Single Institutional Series of 253 Cases

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Objectives: This study aimed to evaluate the role of FNA biopsies in the detection of common and uncommon lesions of the pancreas in a single institutional series.

Materials and Methods: A computer database search was conducted for all pancreatic FNAs completed between January 1996 and October 2015. The cases were analyzed for FNA cytological diagnosis, patient age, and gender with histological correlation as available. Microsoft Excel was used for data entry and analysis.

Results: A total of 253 cases were identified of which 84.6% (214) yielded diagnostic material. The mean age of the patients was 66.2 years old (range 19 to 91), with 52.6% (133) females and 47.4% (120) males. There were 77 benign diagnoses, 105 malignant lesions, and 32 suspicious or atypical specimens. Definitive histology available in 61/253 (24.1%) cases had an 86.9% (53/61) diagnostic accuracy. In almost all cases, the cytopathological and immunocytochemical findings both in the slides and cellular blocks along with the radiological correlation allowed for a precise diagnosis with advanced malignant cases not proceeding for any additional biopsy or resection. Adenocarcinoma and neuroendocrine lesions were identified in 94/105 (89.5%) and 8/137 (5.8%) of cases respectively. The sensitivity for diagnosing a malignant lesion was 87.8% and the specificity was 72.7%. Discordant results included 5 cases of adenocarcinoma on histology diagnosed as negative for malignancy on FNA (presumed sampling error), chronic pancreatitis rather than low grade neoplasm (interpretive error), normal pancreas rather than adenocarcinoma (presumed sampling error), and low-grade cystic mucinous neoplasm rather than high grade neuroendocrine lesion (interpretive error). Nine uncommon lesions identified on FNA included pancreatic tuberculosis, lymphoepithelial cyst, solid pseudopapillary neoplasm, intraductal papillary mucinous neoplasm, oncocytic type with high-grade dysplasia, gastrointestinal stromal cell tumor and metastases from esophageal squamous cell carcinoma, rhabdomyosarcoma, adenosquamous carcinoma and renal cell carcinoma.

Conclusion: An increasing trend of FNA biopsies of the pancreas is attributed to the use of endoscopic ultrasound in the last few years that has greatly improved the yield of diagnostic samples with higher accuracy close to 90% and sample adequacy with diagnostic concordance. We recommend FNA as an easy, safe, reliable, cost-effective investigative tool in the evaluation of common and uncommon pancreatic lesions with a high diagnostic accuracy.

Disclosure of Interest: None declared.

O-054

Needle Tract Seeding in the Gastric Wall by Preoperative Endoscopic Ultrasound Guided Fine Needle Aspiration of Pancreatic Adenocarcinoma: A Case Report and Review of the Literature

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Objectives: Needle tract seeding of the tumor cells via endoscopic ultrasound guided fine needle aspiration (EUS-FNA) of pancreatic masses have been reported sporadically in the literature. Recently we experienced postoperative recurrence of a pancreatic adenocarcinoma on the gastric wall in a patient who had undergone preoperative EUS-FNA via trans-gastric approach.

Material and Methods: A 79 years old man who had been followed for intraductal papillary neoplasm of the bile duct for 2 year was found to have a mass lesion in the body of the pancreas on CT scan. After EUS-FNA of the mass through the gastric wall, a diagnosis of pancreatic adenocarcinoma was made and distal pancreatectomy was performed. Microscopic examination revealed 16 mm moderately differentiated adenocarcinoma of the pancreas with negative resection margins. No lymph node metastasis was identified. Postoperative course was uncomplicated and the patient was discharged.

Result: Twelve months after the surgery, the patient was found to have a mass lesion in the gastric wall on the CT scan, which had not been present 6-month after the surgery. EUS-FNA of this mass was performed to show adenocarcinoma. Histologic and immunohistochemical study including MUC1, MUC2, MUC5AC, and MUC6 showed identical findings between the patient's resected pancreatic mass and the lesion in the gastric wall. The location of the gastric mass was consistent with the needle track of the preoperative trans-gastric EUS-FNA.

Conclusion: Needle tract seeding is uncommon, but real complication of EUS-FNA. We should be aware of the risk and benefit of this procedure and prudentially select the application and the appropriate approach for each pancreatic mass.

Disclosure of Interest: None declared.

O-055

Immunohistochemical Analysis of Pancreatic Cancers by Using EUS-FNA Samples

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Objectives: The prognosis of pancreatic cancers is extremely poor due to a dearth of early diagnostic procedures. Cytological diagnosis of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) can provide valuable information for the diagnosis of pancreatic cancers; however, it is often difficult to make accurate

diagnosis using EUS-FNA samples because sometimes only a few malignant cells are present in the aspirate. This study aimed to investigate the usefulness of immunohistochemical analysis using EUS-FNA samples for the diagnosis of pancreatic cancers.

Methods: We retrospectively evaluated 60 consecutive cases of EUS-FNAs performed between 2013 and 2015 at our hospital. We performed immunohistochemical analysis for expression of MUC1, MUC2, MUC5AC, MUC6, p53, and MIB1 using cell blocks of EUS-FNA samples, and compared their expression in cases with and without cancers. Additionally, we performed immunohistochemical analysis on surgically resected samples (n = 5), and compared the results with that of EUS-FNA samples.

Results: Compared to cases without pancreatic cancers, cases with pancreatic cancers showed increased levels of MUC1. MUC5AC expression in cases with pancreatic cancers was lower than in those without pancreatic cancers. Most cases were negative for MUC2 and MUC6, and their expression did not show variation between the two sample sets. A statistically significant increase in MIB1 expression was observed in cases with pancreatic cancers compared to that in cases without pancreatic cancers. Expression of p53 showed a similar tendency to MIB1; however, it was not statistically significant. The expression levels of mucins showed a variety of ranges when compared between EUS-FNA samples and surgically resected samples obtained from the same individual. Positivity of MIB1 and p53 showed a similar tendency between EUS-FNA samples and surgically resected samples obtained from the same individual.

Conclusions: Our results suggest that immunohistochemical analysis using cell blocks obtained by EUS-FNA is a useful tool to make accurate diagnosis for pancreatic cancers.

Disclosure of Interest: None declared.

O-056

Early Detection of Pancreatic Ductal Intraepithelial Neoplasia (Mild Dysplasia to Carcinoma) by Cytological Evaluation Using Nuclear Bulging Sign (NBS)

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Objectives: For early detection of pancreatic ductal cancer, we have shown a novel cytological method using Nuclear Bulging Sign (NBS), and three cases of early pancreatic ductal carcinoma diagnosed by this method.

Material and Methods: We have shown three cases of early pancreatic ductal cancer. All three cases had been followed by Cytology, ERCP and UES. NBS check was done under the high power field by light microscopy. Cytological sapmles were stained by Papanicoloau's method. NBS is nuclear three dimensional abnormality that caused by abnormal distribution of intranuclear substances, and have benn found in all the cell of malignant cell lines (preneoplastic to invasive carcinoma).

Result and Conclusion: Three cases of early pancreatic ductal cancer have been diagnosed. NBS has been effective to detect the early intraepithelial neoplasia (early IN) and carcinoma. To detect early cancer, it has been critical to detect early stage IN. NBS has been effective to differentiate not only true IN and normal epithelium, but also carcinoma cells and regenerative cell.

Disclosure of Interest: None declared.

O-057

Cytological Difference of Nuclei between Hepatocellular Carcinoma and Cholangiocellular Carcinoma

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Objectives: Utility of cytology for hepatic or bile duct nodules involving hepatocellular carcinoma (HCC) and cholangiocellular carcinoma (CCC) is quite restricted. Especially, tumor cells of HCC are rarely obtained as diagnostic materials of cytology. Although little knowledge have been accumulated for cytology of HCC, clinical situation which requests differential diagnosis of HCC and CCC is rarely presented. In this study, we analyzed 47 hepatic or bile duct nodules of stump cytology focusing on morphology of nuclei and compared HCC with CCC.

Materials and Methods: 47 hepatic or bile duct nodules of 41 surgical cases in Saga University hospital between 2010 and 2012 were involved in this study. Stump cytology was performed by surgically resected specimens. The details of the cases were HCC: 39 nodules (well differentiated: 8, moderately differentiated: 29, poorly differentiated: 2) and CCC: 8 nodules. Cytology of non-tumor cells in background liver were obtained from 5 cases. Nuclei of tumor cells were analyzed by Tissue Studio (Definiens) in samples stained by Giemsa stain and by EXpath III (INTEC) in samples stained by Papanicolaou stain, respectively.

Results: In Papanicolaou staining, means of major axis, means of minor axis, major axis/minor axis ratio and area of nuclei were following, HCC/CCC: 8.1/11.0 μm ($P < 0.0001$), 6.1/6.4 μm ($P = 0.67$), 1.33/1.79 ($P < 0.0001$) and 66.7/78.8 μm^2 ($P = 0.60$), respectively. In Giemsa staining, means of major axis, means of minor axis, major axis/minor axis ratio and area of nuclei were; HCC/CCC: 13.4/14.6 μm ($P = 0.64$), 10.3/11.2 μm ($P = 0.64$), 1.33/1.35 ($P = 0.78$) and 107.3/124.7 μm^2 ($P = 0.57$), respectively.

Conclusion: Nuclei of CCC are significantly larger and oval-shaped than nuclei of HCC in Papanicolaou staining. These features would be useful information in differential diagnosis of HCC and CCC by cytology. However, no significance of these features is observed in Giemsa staining.

Disclosure of Interest: None declared.

O-058

A Case of Inflammatory Hepatocellular Adenoma Displaying an Unusual Pattern

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Background: Cytological diagnosis of hepatocellular adenoma (HCA) is difficult, since it is a very rare tumor and lacks characteristic cytological features. Although rare, HCA contains some unusual histological variants. We have just reported a case of inflammatory HCA displaying an unusual histological pattern (Clin. J. of Gastroenterology, 2015. DOI 10.1007/s12328-015-0614-7). From the operation specimen of this case, touch cytology smear was obtained, which show very unique figure.

Case: A 56-year-old male underwent partial hepatectomy for 1.4 cm sized lesion in the right posterior lobe of liver detected by following up CT for double cancer of stomach and esophagus operated two years before. He had never suffered from glycogen storage disease, diabetes mellitus or obesity.

(Histological findings) The tumor consisted of two types of cells. The first cells, which were designated the common adenoma cells, were larger and exhibited eosinophilic granular cytoplasm, large nuclei and apparent nucleoli. These cells show strong positivity for Serum amyloid A (SAA) and C-reactive protein (CRP). These were compatible with inflammatory HCA. The other cells, which were designated the pyknotic cells, were smaller and demonstrated homogenous more eosinophilic cytoplasm and pyknotic nuclei without nucleoli. These cells were negative for SAA or CRP. In addition, they express cleaved caspase-3 and FAS ligand. The pyknotic cells might be in apoptotic process, which might be induced by FAS-FASL system with mitochondrial pathway.

(Cytological findings) Cytology material shows 1. Although 'inflammatory' HCA, there is small amounts of inflammatory cells 2. HCA cells show characteristic two cell patterns as histological finding. Common adenoma cells contain thick cytoplasm and high N/C ratio with well-defined cytoplasmic border. Nucleoli are frequently seen. The pyknotic cells show small pyknotic nucleus with low N/C ratio. Nucleoli are not frequently seen. Difference between both cells was emphasized in cytological material, since these cells are seen in scattered form.

Conclusion: We report new rare subtype of inflammatory HCA. Cytological diagnosis of this type of variant could be made for precise diagnosis in fine needle aspiration cytology since cytological findings are so characteristic.

Disclosure of Interest: None declared.

Thyroid 1

O-059

Performance of Next-Generation Sequencing on Thyroid FNA Smears: A Retrospective Study on 53 Cases

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Objectives: Thyroid FNA is a cost-effective tool to triage patients for either thyroid nodule removal or follow up. However, patients with 'undeterminate' cytology (AUS/FLUS, FN/SFN and Suspicious for Malignancy Bethesda classes) may undergo unnecessary surgery for benign nodules. A growing number of molecular tests that identify genetic alterations associated with thyroid malignancy, may help the cytopathologist in the risk stratification of indeterminate cytological diagnoses. Among these tests, the Next-Generation Sequencing (NGS) enables the assessment of many genetic alterations at same time on a small amount of DNA, such as that obtained from cytological specimens. The aim of this study is to assess the feasibility of NGS on a retrospective series of 53 thyroid FNAs.

Materials and Methods: The laboratory information database of the study institution was searched to obtain the diagnostic reports recorded in the system from January 2010 to December 2011 related to thyroid US-guided FNAs. Fiftythree Diff Quik stained smears with the following cytological diagnoses were selected for NGS analysis on Ion Torrent Platform by using the HotSpot Cancer Panel: n = 3 AUS/FLUS, n = 11 FN/SFN, n = 12 SFM, n = 27 Malignant. Results obtained from n = 28/53 (52%) samples were validated by an independent orthogonal platform (pyrosequencing). In n = 23/53 cases (43%) NGS analysis was performed also on the histological matched samples.

Results: n = 41/53 (77%) of samples reached an overall adequate amplicon coverage (DNA yield, range 0.42–35.5 ng/μl, mean 6.10). A lower DNA yield was obtained from samples that have failed the NGS analysis (n = 12/53, 22%; DNA yield range 0.26–14.3 ng/μl, mean3.12). Conversely, no differences in cellularity was observed between adequate and failed samples. The Ion Torrent Variant Caller Software called n = 22/41 BRAF mutations, n = 1/41 NRAS mutations, n = 2/41 KRAS mutations, n = 4/41 KIT mutations and 1/41 RET mutation. On the overall, pyrosequencing confirmed all the NGS variant calls; only n = 1/22 BRAF mutation was not confirmed. This latter case, however, showed both low DNA quality and coverage. On matched histologies, NGS showed overlapping results; only 1 BRAF mutation was not confirmed due to low amplicon coverage on histology. N = 6/41 cases were wild type by NGS. However, one of these latter cases was a false negative: it showed an inadequate BRAF amplicon coverage on NGS, while BRAF mutation was detected by pyrosequencing.

Conclusion: NGS is feasible on routine thyroid FNAs. However, orthogonal molecular techniques should be available to confirm unsufficiently covered wild-type amplicons and low frequency variants.

Disclosure of Interest: None declared.

O-060

BRAF V600E Mutation Detection by ARMS-PCR on DNA Extracted from Scraped Smears and from Direct Sample: Diagnostic Utility in Thyroid FNA

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Objective: A proportion of papillary thyroid carcinoma (PTC) display the BRAF V600E mutation which is a molecular marker for this tumor. The objective of this study was to i) evaluate its diagnostic utility in thyroid FNA with special reference to the AUS/FLUS category and ii) evaluate feasibility of using cell scrape DNA for molecular testing.

Materials and Method: A total of 85 cases including 40 PTCs, 3 suspicious for malignancy, 28 AUS/FLUS, 14 benign (6 thyroiditis and 8 colloid goiters) were evaluated for the BRAF V600E mutation by the Amplification Refractory Mutation System-(ARMS)-PCR previously described. The DNA for molecular testing was extracted from the direct sample collected at the time of FNA in RNA later (Ambion) in 50 cases and from smear scrapes of 1–2 conventional air-dried Giemsa stained smears in 35 cases. The internal control band of 200 bp was required for a test sample to be interpreted as positive. Mutant BRAF was represented by a 144 bp band and wild type BRAF by a 98 bp band.

Results: Out of 85 samples, interpretation was possible in 71 cases. There was no difference in the interpretability of direct vs. scrape smear sample. Overall, BRAF V600E mutation was positive in 20 cases. After categorization, we observed that 16/31 (52%) conventional PTC, 2/5 (40%) cystic PTC and none of the PTC with Hurthle cell change (3) of harbored BRAF V600E mutation. All 3 cases suspicious for malignancy were negative for the mutation. 2/17 cases (12%) of AUS/FLUS showed mutant BRAF, one of which was confirmed on histology to be a PTC; follow-up was not available in the other case. All 14 benign lesions including colloid goiter and thyroiditis were negative for mutation.

Conclusion: Molecular testing for BRAFV600E mutation by ARMS-PCR is a useful and simple test that can be performed equally well on DNA extracted from cell scrapes and direct samples and provides immediate results. The sensitivity for detecting PTC was 47% and specificity was 100%; however, in AUS/FLUS category it showed poor sensitivity.

Disclosure of Interest: None declared.

O-061

Galectin-3 and CK19 Expression on the Cellblocks from FNA in Differential Diagnosis of Thyroid Lesions

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Introduction: Fine needle aspiration cytology has been recognized as good modality of choice for the thyroid-nodule evaluation. Galectin-3 marker was suggested as an indicator of malignancy and a potential tool in the differential diagnosis of the follicular patterned of thyroid lesions. Galectin-3 and CK19 are highly expressed in thyroid carcinoma but not in normal thyrocytes and in benign lesions such as follicular adenoma, can be used as combination for distinguishing benign from malignant lesions, as sensitive markers that enhance the diagnostic accuracy of FNA in thyroid lesions.

Aims and Objectives: 1. To evaluate the immunocytochemical expression of Galectin-3 and CK-19 on cellblock of thyroid lesions.

2. To study the diagnostic accuracy of Galectin-3 and CK-19 expression on cellblocks, and Bethesda System for reporting in FNA cytology with histopathological correlation of thyroid lesions.

Materials and Methods: 106 patients diagnosed with thyroid lesions on FNA cytology were studied prospectively (2012–2014) with cellblocks. Immunocytochemical expression of Galectin-3 and CK19 was performed on cell blocks for each case. The pattern of expression for this marker (cytoplasmic or nuclear) in cells and its intensity were noted. Sensitivity and specificity were calculated taking histopathology as gold standard.

Results: The cellblocks from FNA from thyroid lesions were obtained from 106 patients (21 males and 85 females), age ranged from 17–70 years. In 65 aspirates, the cytological diagnosis was benign lesions (comprising of colloid nodule, hyperplastic nodule, hashimoto's thyroiditis and adenoma). Galectin-3 expression in all benign cases showed negative immunostainig (cytoplasmic or nuclear) except 2 cases (weak intensity as 1+). In 40/41 malignant cases, Galectin-3 expression was reported as strongly and highly positive (4+, 3+) cytoplasmic or nuclear immunostaning as scoring or intensity in more than 10 percentage distribution in tumor cells. In 1/41 malignant cases, Galectin-3 epxpression was negative or showed poor staining (1+). CK19 expression in all benign cases showed negative immunostainig (cytoplasmic or nuclear) except 4 cases (positive scoring and intensity as 1+ or 2+). In 39/41 malignant cases, CK19 expression was reported as strongly and highly positive (4+ or 3+) cytoplasmic or nuclear immunostaning. In 2/41 malignant cases, CK19 epxpression was poor staining or focal staining (1+ and 2+). The sensitivity and specificity of Galectin-3 and CK19 on cellblocks as a marker for thyroid malignancies were 98.0%, 100% and 95.1, 100% respectively in our study.

Conclusion: Galectin-3 and CK19 immunocytochemistry were found to be excillent useful reliable markers on cellblocks from FNA in differential diagnosis of the thyroid lesions.

Disclosure of Interest: None declared.

O-062

Two-Photon Live-Cell Imaging Discloses Diagnostic Cytomorphology and Novel Cellular Dynamics in Living Human Papillary Thyroid Carcinomas.

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Objectives: Two photon microscopy provides a high-resolution image deep within living tissue fragments, with minimal phototoxicity. In a first application of two-photon microscopy for cytology, we established conditions for live cell imaging of the nuclear features of a living papillary thyroid carcinoma (PTC) in intact FNA-sized tissue ex-vivo, and observed unexpected nuclear dynamics.

Materials and Methods: FNA-sized fragments of a freshly excised human PTC were stained for 1 minute in 0.01% acriflavine (Sigma) in Hanks saline, mounted on a 50 mm coverslip, anchored with polyester mesh in a flow-through channel, and perfused with fresh Hanks in a two photon microscope built by M.J.S. which includes an insulated chamber heated to 37 degrees.

Result: Acriflavine penetrates live cells, binds to nucleic acids, and fluoresces strongly with a peak of about 540 nm with two photon excitation in the infrared at 795 nm. The acriflavine emission can be inverted and pseudocolored to look very similar to hematoxylin. Morphologic features of cell death were not seen during 30 minutes of imaging, suggesting minimal phototoxicity. Cell viability can be monitored by infusing ethidium bromide which is excluded from viable cells and has a separate emission spectrum from acriflavine. Diagnostic features of PTC were seen in living cells, including dispersed heterochromatin, nuclear grooves, and intranuclear cytoplasmic inclusions. In monolayer cultures using phase contrast microscopy, we previously observed a dynamic shuttling of the nucleus of PTC cells within the cytoplasm. Using two-photon microscopy of PTC cells within a tissue microenvironment, a similar behavior was observed at higher resolution, with a rapid hob-nail-like back-and-forth movement of the whole nucleus within the cytoplasm, over a span of about three minutes. Preliminary observations suggest that rotation of the nucleus within the cytoplasm is an unexpected dynamic diagnostic feature of thyroid follicular neoplasms that distinguish them from normal thyroid epithelial cells.

Conclusions: The diagnostic nuclear features of PTC are not artifacts of cytopreparation since they are seen in living cells. Two photon microscopy has many potential applications in cytology. Live cell imaging shows cellular dynamics that cannot be anticipated based on our >100 year experience with static diagnostic images. These dynamics likely have diagnostic utility and may provide important clues about the molecular mechanisms of cancer. Abnormalities of the known connections between the nuclear lamina and the cytoskeleton are unexpectedly implicated in thyroid cancers based on these observations.

Disclosure of Interest: None declared.

O-063

Cytopathologist-Performed Ultrasound (US)-Guided FNA of Thyroid Nodules: Use of the 2015 American Thyroid Association (ATA) US Guidelines and Impact in the Cytological and Molecular Evaluation

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Objectives: Emphasize:

1) The positive diagnostic impact of US-FNA performed by the cytopathologist,

2) The value of the 2015 ATA US guidelines to select thyroid nodules suitable for molecular tests, and

3) The importance of Cytologic-Sonographic-Molecular correlation for patient management.

Materials and Methods: All patients were seen in our outpatient clinic in 2015.

After US evaluation, US-FNA of thyroid was performed with 27-g needles using the Zajdela technique, 3 passes performed, and 3 smears prepared. Giemsa-stained smears were read and diagnosis made in 24hs. The 2015 ATA US rates of suspicion were assessed at time of US to triage nodules and harvest material for molecular tests in selected cases (only since June 2015). Based on the US pattern, suspicion was rated as high, intermediate, low, very low, or benign. After reading all Giemsa-stained smears, we evaluated the need to send the material for molecular tests, which included: oncogene mutations and gene rearrangements/translocations, and a panel of microRNA markers to discriminate benign vs. malignant disease.

Results: We performed 1499 US-FNA of various organs in 2015. 982 (65.5%) were thyroid nodules, 5 (0.3%) parathyroid tumors, 5 (0.3%) thyroglossal duct cysts, and 507 (33.8%) miscellaneous cases. The Bethesda System (TBS) was used to report thyroid nodules: TBS1 1.4%, TBS2 87.2%, TBS3 2.0%, TBS4 3.7%, TBS5 1.2% and TBS6 4.5%. Ratio indeterminate diagnoses (TBS3-5)/TBS6 = 1.5.

We sampled 100 nodules for molecular tests (often intermediate and high suspicion US patterns). Molecular tests were performed in 25 (TBS3 4, TBS4 19, TBS5 1, and TBS2 1). 18 (72%) had no molecular abnormalities (TBS2 1, TBS3 4, TBS4 12, and TBS5 1). Based on cytology and US findings surgery was performed in 1 TBS4 and 1 TBS5 (papillary carcinoma in both). 7 (28%) (all TBS4) had molecular abnormalities and on short follow-up: follicular adenoma (2), scheduled surgery (3), and no surgery (2). Final diagnoses were amended based on the Cytologic-Sonographic-Molecular correlation. Recommendations were discussed with the treating physician.

Conclusions: 1. Our experience supports that US-FNA performed by experienced cytopathologists reduces unsatisfactory (1.4%) and inconclusive (6.9%) rates.

2. Intermediate and high US suspicion patterns strongly correlate with TBS3-6.

3. 2015 ATA US guidelines at the time of US-FNA help to select the nodule and obtain material for possible molecular tests.

4. Judicious use of molecular tests helps to further assess the risk of malignancy in TBS3-5 diagnoses.

5. In some cases (2/25, 8%), cytological diagnosis prevails over negative molecular test results and directs surgical approach.

6. Cytologic-Ultrasound-Molecular correlation helps in patient management.

Disclosure of Interest: None declared.

O-064

Thyroglobulin Measurement of Aspirated Fluid Improved the Detection of Cystic Metastatic Thyroid Papillary Carcinoma

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Background: Cystic change in the metastatic lymph node of thyroid papillary carcinoma (PTC) may be a diagnostic challenge for fine needle aspiration cytology (FNAC) due to scant cellularity. The aim of this study was to evaluate the thyroglobulin level in FNA (Tg-FNA) for detecting metastatic PTC in patients with cystic neck lesions and to validate the optimal cutoff value of Tg-FNA.

Methods: All cystic neck lesions with or without the history of PTC were performed both FNA and Tg-FNA examinations. The cytological findings, Tg-FNA concentrations, and follow-up of histology and imaging were correlated. We evaluated diagnostic accuracy of Tg-FNA in metastatic PTC detection according to several predetermined threshold levels: minimal detection level of Tg measurement (0.04 ng/mL), 0.9 ng/mL, 10 ng/mL, and maximum normal level of serum-Tg (77 ng/mL).

Results: A total of 75 FNA specimens of cystic lesions were identified, including 40 metastatic PTC. The area under the ROC curve of Tg-FNA value in determining metastatic PTC at 0.04, 0.9, 10 and 77 ng/ml was 0.5 (95%CI, 0.382–0.618), 0.645 (95%CI, 0.526–0.752), 0.945 (95%CI, 0.866–0.984) and 0.973 (95%CI, 0.907–0.996) respectively. Using a cutoff value of 77 ng/mL, Tg-FNA and FNA cytology combining strategy showed superior diagnostic power (97.5% sensitivity and 100% specificity) when compared with diagnostic strategy FNA cytology alone (80% sensitivity and 100% specificity).

Conclusions: Tg-FNA is a useful ancillary test that improves the detection of cystic PTC metastases. We recommend that the threshold values for Tg-FNA levels in cystic neck lesions should be the maximum normal serum-Tg level (77 ng/mL).

Disclosure of Interest: None declared.

O-065

Malignancy Rate in Thyroid Nodules Categorized as Atypia of Undetermined Significance (AUS) or Follicular Lesion of Undetermined Significance (FLUS) – An Institutional Experience

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Objectives: Atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS), category III of Bethesda system for reporting thyroid cytopathology (BSRTC) has emerged as most controversial category due to its heterogeneity and inconsistent usage. Initially associated risk of malignancy was estimated to be about 5% to 15% but eventually different results have been obtained across institutions due to variable follow ups and interpretation. The present study was conducted to evaluate the rate of malignancy in category III of AUS/FLUS of BSRTC in light of clinical and radiological features in our institute.

Material and Methods: All the cases diagnosed as AUS/FLUS, category III on BSRTC over period of five years were included in the study. Detailed clinical history, biochemical and radiological examination including thyroid ultrasonography reports and thyroid scans, wherever available were recorded along with follow ups and management of all cases. Histopathology was available for cases which underwent surgery due to high clinical suspicion, worrisome radiological features or two consecutive AUS/FLUS reports on repeat fine needle aspiration cytology (FNAC). Cases which did not undergo surgery were followed up for at least one year.

Results: Out of total 1325 thyroid FNAC, 88 cases were categorized under AUS/FLUS with incidence of 6.6%, mean age of 49.5 years, female preponderance (2.8:1) and mean nodular size of 2.2 cm. Worrisome ultrasonographic features were observed in 22.8% of cases including hypervascularity, hypoechoic complex nodules and microcalcifications. Repeat FNAC was done in 38.6% cases (34/88), immediate surgery in 48.8% cases (43/88) and follow up without any intervention in 12.5% cases (11/88). Repeat FNAC observed 23.5% cases as malignant, 67.6% benign and 8.8% (3/34) were again categorized as AUS/FLUS which after surgery turned out to be malignant neoplasm. In cases undergoing immediate surgery 72% (31/43) were benign lesions and 27.9% (12/43) were malignant on histopathology. One case on observational follow up was subjected to surgery due to sudden increase in nodular size which turned out to be malignant. The overall malignancy rate was 27.2% out of total cases diagnosed as AUS/FLUS (24/88) with most common malignancy being classical papillary carcinoma followed by follicular carcinoma and follicular variant of papillary carcinoma.

Conclusion: Bethesda category III of AUS/FLUS on thyroid cytology may be associated with higher risk of malignancy in comparison to initial estimations. Surgical intervention may have greater implication than repeat FNAC or observational follow-ups in appropriate management of this heterogeneous category.

Disclosure of Interest: None declared.

O-066

Malignant Risk in Patients with Thyroid Nodules Diagnosed as Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS) in Fine Needle Aspiration (FNA) – Does Repeated FNA Really Play a Crucial Role in Management?

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Objects: To evaluate benefits in predicting malignancy in repeated thyroid fine needle aspiration (FNA) in patients previously classified as 'atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS)', and to determine if any clinical or sonographic features predicting a higher malignant risk in those patients.

Material and Methods: A total of 1735 thyroid FNAs in 1292 patients were performed in Koo Foundation Sun Yat-Sen Cancer Center from 2013 to 2014, and 277 (16.0%) aspirations in 234 patients were diagnosed as AUS/FLUS. Tracing the history of these 234 patients, 220 (94.0%) underwent thyroid FNA twice or more, including 60 cases found AUS/FLUS during their follow-up of underlying benign thyroid nodules, and 160 cases diagnosed as AUS/FLUS initially, while 14 (6.0%) underwent FNA just one time before definitive surgery. We classified these patients as *group 1* (AUS/FLUS-diagnosed once, 122 patients), *group 2* (AUS/FLUS-diagnosed twice or more, 83 patients), and *group 3* (AUS/FLUS, followed or accompanied by (suspicious for) malignancy or follicular neoplasm, 29 patients). A total of 83 patients received definitive surgery, comprising 18.0% (22), 44.6% (37), and 82.8% (24) in the three groups, respectively.

Result: Malignancy comprised 63.6% (14/22), 51.4% (19/37), 87.5% (21/24) in the three groups, respectively, with a p-value of 0.015. However, there was no significant difference statistically between *group 1* and *2* or between *group 1* and *group 2+3* (data combined), demonstrating few additional benefits of repeated FNA. As for the clinical course of nodules, patients with an initial FNA result of AUS/FLUS seemed to have a higher risk for malignancy than those who were found AUS/FLUS during their follow-up for underlying benign nodules, but no significant difference was demonstrated (69.7% vs. 47.0%, p-value = 0.081). Other clinical features, including gender, age, tumor size and multifocality, as well as sonographic categories showed no significant difference between benign and malignant cases.

Conclusion: Although the interpretation of FNA cytology is pathologist-dependent, AUS/FLUS group certainly indicates an intermediate risk of malignancy. Routinely repeated FNA in these patients has limited efficacy. Management is sometimes difficult and challenging. Clinicians should take all of the clinical information, sonography and cytology result into consideration.

Disclosure of Interest: None declared.

O-067

'Borderline' Atypical Cytological Features and Low Cellularity in Thyroid Fine Needle Aspiration Cytology (FNAC) Resulting in Difficulty in Accurate Diagnosis of Follicular Variant of Papillary Thyroid Carcinoma (FVPTC) Preoperatively

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Objectives: To understand the reasons why follicular variant of papillary thyroid carcinoma (FVPTC) is often diagnosed as atypia of undetermined significance/follicular lesion of insignificance (AUS/FLUS) in preoperative fine needle aspiration cytology (FNAC) and to validate the usefulness of some well-known diagnostic features.

Materials and Methods: Twenty-eight patients were diagnosed as FVPTC after receiving thyroidectomy in Koo Foundation Sun Yat-Sen Cancer Center (KFSYSCC) in 2014. Thirty-nine FNACs were performed in these patients preoperatively, and 1 (2.6%) undiagnostic, 3 (7.7%) benign, 20 (51.3%) AUS/FLUS, 4 (10.3%) suspicious for follicular neoplasm, 6 (15.4%) suspicious for malignancy, 5 (12.8%) (FV) PTC were diagnosed. All of the cases were reviewed by a pathologist and a cytotechnologist blindly. Cytological features were evaluated and recorded quantificationally, including nuclear features (chromatin pattern, nuclear grooving, pseudoinclusion, small nucleoli, anisonucleosis and irregular nuclear membrane), cellularity, microscopic pattern (microfollicles, monolayered sheets, papillary fronds), features of colloid, and lymphocytes or hemorrhage in the background.

Result: Among the 28 cases, 11 underwent twice thyroid FNA before surgery. The mean diameter of resected tumors was 2.2 cm, and 8 cases were of multifocality. A total of 192 smears were reviewed (average 5 slides/FNAC), and 61.5% cases remained the same diagnosis. Most of the reclassified cases were upgraded to the suspicious group. However, diagnosis of the AUS/FLUS group was indeed based on borderline degrees of following cytological features statistically with a significant difference, including nuclear features such as nuclear grooving, pseudoinclusion, pale chromatin, irregular membrane and anisonucleosis, and microscopic patterns of microfollicles, and cellularity. Colloid features (watery or dense), the background of hemorrhage or lymphocytic infiltration and presence of nucleoli were not helpful in making a diagnosis more accurately in FNA.

Conclusion: The high tendency of being classified as AUS/FLUS group in FVPTC attributes to their 'borderline' cytological features. Examiners should be more cautious about missing these

inconspicuous clues and making a false-negative diagnosis. Since cellularity is a key criterion for evaluation and FNA is an operator-dependent technique, smears with higher quality in cellularity help us not to under-diagnose FVPTC cases in FNAC.

Disclosure of Interest: None declared.

O-068

Risk of Malignancy and Risk of Neoplasia in the Bethesda Indeterminate Categories: Study on 4532 Thyroid FNA from a Single Institution in India

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Objectives: The category 3, 4 and 5 of the Bethesda system of reporting thyroid cytopathology (TBSRTC) together constitute the indeterminate thyroid nodule (ITN) with an increasing risk of malignancy (ROM). The aim of this study was to evaluate our indeterminacy rate and risk of malignancy and neoplasia in the different Bethesda categories. Secondary objective was to determine the diagnostic accuracy.

Materials and Method: The Bethesda system for reporting thyroid cytopathology (TBSRTC) was used to perform a retrospective 6-year audit of 4532 thyroid FNA from a single institution. Histological correlation was available in 335 cases overall and in 54 cases of ITN.

Results: The overall rate of AUS/FLUS was 2.5% with an increasing trend in last 2 years reaching 5%. Category 4 rate showed less variation across the years averaging 3.9%. Category 5 rate showed an increasing trend averaging 0.5%. The upper bound estimates of the risk of malignancy in Category 3, 4 and 5 were 58.3%, 23.6% and 75% and risk of neoplasia was 66%, 65.7% and 100%. AUS/FLUS (N = 116 cases) was sub-categorized as AUS (7), AUS-HC (Hurthle cell, 5), AUS-PTC (25), FLUS (49), FLUS-FH (favor hyperplasia, 13), FLUS-HC (6) and FLUS-PTC (FVPTC favored, 11). Upon histological correlation (n = 11), FLUS category carried high risk of malignancy (4/5), with too few cases in the other categories to draw conclusion. Cystic papillary carcinoma, distinction between follicular hyperplasia and neoplasia, thyroiditis with Hurthle cell hyperplasia and non-specificity of nuclear changes of papillary thyroid carcinoma were sources of diagnostic errors. If indeterminate categories were considered as positive cytological diagnosis, then the sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of FNA thyroid was 80.5%, 85.9%, 80.7%, 85.7% and 82.4% respectively.

Conclusion: The introduction of TBSRTC has led to an increasing trend in diagnosis of AUS/FLUS lesions which are still managed conservatively in most cases. In view of the high risk of neoplasia and of malignancy in the AUS/FLUS category in our setup, surgery should be considered as the best option for management of TBSRTC category 3 rather than conservative approach. Overall, thyroid FNA has a good diagnostic accuracy in our institution.

Disclosure of Interest: None declared.

Cervix 2

O-069

Follow-Up of Atypical Squamous Cells, Cannot Exclude High Grade Intraepithelial Lesion ASC H, and Significance of High Risk Human Papillomavirus DNA in Triage of ASC H

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Objectives: Atypical squamous cells cannot exclude high grade squamous intraepithelial lesions ASC H is a nomenclature of the Bethesda System. The objective of this study was to investigate the histological results of ASC H for three year follow up of a cohort of women at clinics of Guangdong general hospital, Guangzhou, China. And to evaluate the clinical significance of high risk human papillomavirus DNA testing in ASC H.

Materials and Methods: Of all 150 cases diagnosed as ASC H on cytology, 108 cases were taken with Colposcopic examination and biopsy over a three year follow up. 89 cases were conducted with high risk HPV DNA testing.

Results: 59 were diagnosed as high grade intraepithelial lesion HSIL and 12 were low grade intraepithelial lesion. HPV DNA was found to be positive in 45 of HSIL, while in 35 cases of HPV DNA negative, 3 cases were HSIL, and 10 were LSIL.

Conclusions: ASC H strongly predicts the presence of HSIL. HR HPV may serve as a predict select whether a patient should take colposcopic examination immediately. Patients with positive HR HPV should undergo immediate colposcopic examination.

Disclosure of Interest: None declared.

O-070

Analysis of Prevalence of Human Papillomavirus Infection in Southwestern China and Its Correlation with Cervical Lesions

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Objective: To study the prevalence of human papillomavirus (HPV) infection rate and genotype distribution in southwestern China and its correlation with cervical lessions.

Methods: 105780 women who visited West China Second Hospital of Sichuan University from September 2011 to April 2015 were accepted the classification of HPV genotypes, of whom 8393 women had cervical biopsy and were divided into four groups according to its histological diagnosis: chronic cervicitis group (2096 cases); LSIL group (1530 cases); HSIL group (3171 cases); cervical carcinoma group (1596 cases; including Cervical squamous cell carcinomas 1473 cases, adenosquamous carcinoma 43 cases, adenocarcinoma 80 cases). The association between HPV genotypes and different cervical lesions was analyzed.

Results: The overall HPV prevalence was 25.10% (26550/105780), of which 20.95% (22160/105780) of the women were infected by High-risk types and 4.15% (4390/105780) by low-risk types. The most common high-risk genotypes were HPV16, 58, 33, 52 and 18. HPV infection rates showed increasing trend with the increased age as well as the severity of cervical lesions ($p < 0.001$). In cervical lesions, the most common genotypes were HPV16, 58, 33, 18 and 52, while in cervical carcinoma group were HPV16, 18, 58, 33 and 52, in HSIL group were HPV16, 58, 33, 52 and 18 and in LSIL group were HPV16, 58, 52, 56, 33.

Conclusions: The most common high-risk genotypes of HPV were 16, 58, 33, 52 and 18, which existed geographical differences from other regions. HPV infection rates showed increasing trend with the increased age. There were also differences of HPV genotypes in different cervical lessions.

Disclosure of Interest: None declared.

O-071

Evaluation of HPV Testing as an Adjunct to LBC in Cervical Cancer Screening

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Objectives: To assess the effectiveness of including HPV testing as an adjunct to liquid based cytology (LBC) in cervical cancer screening.

Methods: Atypical squamous cells of undetermined significance (ASC-US) were classified according to the 2003 Bethesda classification system. The study ran for 11 years from May 2004 to November 2014 in conjunction with public cervical cancer screening for Kanazawa City resident. During first 7 years, HPV testing was performed as an adjunct to conventional cytology (CC) (2004~20010; HPV with CC).

For the last 4 years, HPV testing was as an adjunct to LBC (2011~2014; HPV with LBC). Patients with ASC-US underwent parallel testing for high-risk HPV types with the Hybrid Capture II system; HPV positive and cytology-ASC-US cases were recalled for colposcopic examination and biopsied. Positive predictive values (PPV; CIN 1 or more/total number of biopsies) were compared with those obtained before HPV testing was initiated (2000~2003; CC without HPV test).

Results: A total of 109,328 women underwent screening over the 11-year period; of these, 6,022 (5.5%) were ASC-US, among whom 1,248 (22.4%) tested HPV positive. These 1,248 women (1.1% of the screened population) were recalled for colposcopic examination. The resulting 1,046 biopsies were diagnosed as CIN 1 (n = 484), CIN2 (n = 116), CIN3 (n = 24), and invasive cervical cancer (n = 6). Before HPV screening initiated (CC without HPV test), the levels of PPV were 58.0 ± 2.9% (mean ± SD). After HPV screening initiated (HPV with CC), the PPV were increased to 66.3 ± 2.3% significantly ($p < 0.05$). As an adjunct to LBC, HPV testing further improved the PPV to 70.6 ± 2.2% ($p < 0.01$).

Conclusion: HPV testing as an adjunct to LBC in cervical cancer screening improves detection sensitivity with proven PPV.

Disclosure of Interest: None declared.

O-072

A Possible Difference in HPV16 and HPV18 Carcinogenesis Arising from the Prevalence of HPV Types in 1374 Japanese Cervical Neoplasia Patients

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Objective: HPV16 and HPV18 have similarity and difference in carcinogenesis of HPV-related cancer, especially cervical cancer. To know the difference between HPV16 and 18, we have addressed the distribution of HPV types in cervical neoplasia using large-scale sample set.

Method: We have done the HPV testing for our 1374 patients in University of Tokyo since 2008 to 2015, that study was approved by an ethical committee in our university. We have collected some samples from their cervix and checked their HPV type by PGMY line blot assay and then a statistical analysis has been done.

Results: 1168 CIN cases (496 CIN3 cases) and 206 cervical cancers were analyzed. In cervical cancer, 81 cases (39%) were HPV16 positive, 39 (20%) cases were HPV18 positive, 16 cases were HPV52 positive, and 7 cases were HPV58 positive. While, in CIN3/AIS (496 cases), 200 cases (40%) were HPV16 positive, 33 cases

(6.7%) were HPV18 positive, 110 cases (22%) were HPV52 positive, and 68 cases (14%) were HPV58 positive. In CIN2 (341 cases), just 76 cases (22%) were HPV16 positive, and 21 cases (6.2%) were HPV18 positive, the other hand 72 cases were HPV52 positive and 64 cases were HPV58 positive. In CIN1 (331 cases) just 26 cases (8%) were HPV16 positive, and 15 cases (4.5%) were HPV18 positive, then 44 cases (13%) were HPV52 positive and 36 cases (11%) were HPV58 positive. A ratio of HPV16 in cervical cancer was equal to that in CIN3/AIS while a ratio of HPV18 in cancer was clearly increased compared with CIN3/AIS.

Conclusion: The prevalence of HPV18-related neoplasia was 4.5% to 6.7% in CIN1-3 whereas 20% in cervical cancer. In turn, the prevalence of HPV16-related neoplasia increased gradually (8, 22, 40, and 39% in CIN1, CIN2, CIN3/AIS, and cancer, respectively). This data suggested that HPV18 may have the potential to jump intraepithelial neoplasia up to invasive cancer.

Disclosure of Interest: None declared.

O-073

Primary HPV Screening in Tampere City, Finland: Genotype Distribution

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Objective: Primary HPV testing has been implemented for cervical cancer screening in the city of Tampere, Finland since 2012 in 35–60 aged females.

Materials and Methods: HPV was assessed with Abbott RealTime High Risk HPV test detecting 14 HPV high-risk genotypes including16 and 18 genotypes. Both HPV test and Pap smear were taken, but only HPV positive pap smears slides were analyzed. HPV genotype results from 2012–2013 screening years are presented with cytology and histology correlations.

Results: HPV was positive in 798 samples out of 11 394 tested females (7%). Genotype 16 was detected in 17% of cases, genotype 18 in 5% of cases and not specified high risk (NSHR) genotypes in the resting 78%. HSIL/ASC-H cytology was revealed in 28/798 samples. 42.5% of them were HPV genotype 16 positive, 7.5% HPV18 positive and 50% NSHR HPV positive. On the other hand, 80% of normal cytology (NILM) samples were NSHR cases, 14% were genotype 16 positive and 6% genotype 18 positive, respectively.

Conclusions: In primary HPV screening HPV16 and HPV18 genotypes were connected with higher grade cytology than NSHR genotypes in agreement with previous research series.

Disclosure of Interest: None declared.

O-074

Distribution of Human Papillomavirus among Women with Abnormal Cervical Cytology in Kuwait

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Objective: This study investigates the distribution of human papillomavirus (HPV) in women with abnormal cervical cytology in Kuwait.

Materials and Methods: 298 abnormal ThinPreps were taken from women seeking routine gynecological care and screened for HPV DNA by real-time PCR. HPV genotyping was determined by PCR-based sequencing.

Results: HPV DNA was detected in 152 women (51%), and 29 different HPV genotypes were detected, comprising 16 high-risk (HR) (16, 18, 31, 33, 35, 39, 45, 51, 53, 56, 58, 59, 66, 68, 73, 97), nine low-risk (LR) (6, 11, 54, 61, 74, 81, 90, 102, 106) and four intermediate-risk (IR) (62, 67, 84, 87). HPV16 had the highest prevalence (24.3%), followed by HPV11 (13.8%), HPV66 (11.2%), HPV33 (9.9%), HPV53 (9.2%), HPV81 (9.2%), HPV56 (7.9%) and HPV18 (6.6%). HPV prevalence was 86%, 67% and 89% in women with invasive cervical carcinoma (ICC), high-grade squamous intraepithelial lesion (HSIL) and low-grade squamous intraepithelial lesion (LSIL), respectively. As for age distribution, 69% of all HPVs were found in women aged 20–29 years, and the HPV incidence rate deceased with increasing age. The proportion of single infections decreased as the severity of the cytological diagnosis increased, while the proportion of multiple infections increased.

Conclusion: This study is the first of its type in Kuwait and one of few in the Middle East. The findings are consistent with the hypothesis that HPV infection is the primary cause of cervical neoplasia. They support HPV vaccine research to prevent cervical cancer and efforts to develop HPV DNA diagnostic tests.

Disclosure of Interest: None declared.

O-075

The Assessment of Cervical Cancer Screening Using the Combination of Cervical Cytology and High-Risk Human Papillomavirus Testing in Fukui Prefecture, Japan

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Objective: The clinical usefulness of high-risk human papillomavirus (HR-HPV) testing in women undergoing routine cervical cytology screening has been debated. The objective of this study was to assess whether the combination of cervical cytology and HR-HPV testing was effective in Japan.

Materials and Methods: For this study, 3473 women aged 25–69 years who were participating in the cervical cancer screening

program by Fukui prefecture in Japan were assigned. All women underwent cervical cancer screening with cytology and HR-HPV testing simultaneously. And women with positive for HR-HPV and negative for intraepithelial lesions or malignancy (NILM) for cervical cytology were referred for colposcopy alone or colposcopy with biopsy in 12 weeks after cancer screening.

Results: The overall prevalence of HR-HPV was 6.9% (n = 238) and of HPV-16/18 was 1.8% (n = 62). The overall prevalence of NILM was 97.1% (n = 3371). 159 women (4.6%) were positive for HR-HPV testing and NILM for cervical cytology and scheduled for colposcopy. Of 159 women, 66 (41.5%) visited our hospital for colposcopy. 59 underwent colposcopy with biopsy and 7 underwent colposcopy alone without abnormal findings. Of 66 women with HR-HPV and NILM for cytology, 6 (9.1%) had cervical intraepithelial neoplasia grade 2 (CIN2) or worse, 13 (19.7%) had CIN 1 and 47 (71.2%) had no dysplastic lesion. Of 6 women with CIN2 or worse, 4 (66.7%) had HPV-16. The overall prevalence of negative HR-HPV was 93.1% (n = 3235). 23 women (0.67%) were negative for HR-HPV testing and no NILM for cervical cytology. Of the cytological results for 23, 10 (43.5%) were inadequate sample, 7 were low-grade squamous intraepithelial lesion (LSIL), 5 were atypical squamous-cells of undetermined significance (ASC-US) and 1 was atypical glandular cells (AGC). No woman with negative for HR-HPV testing had abnormal cytological findings to suspect CIN2 or worse.

Conclusion: This study suggests the possibility that the combination of cervical cytology and HPV testing is effective in Japan. However, if all women with positive for HPV testing and NILM for cervical cytology undergo colposcopy, our data shows that the number of colposcopies without necessary (potential harm) might increase. Therefore we need to find the best way to use HR-HPV testing, which has a good balance between benefit and potential harm.

Disclosure of Interest: A part of this research was funded from Roche Diagnostics company.

O-076

Morphological and Spectral Markers of Cervical Cancer Cells

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Background: Cervical cancer is the second most common cancer in women worldwide. More than 80% of cervical cancers occur in the developing world where the least resources exist for management. Most cases of cervical cancer can be prevented through screening programs. One of most popular program based on Pap-test to reveal bulk properties of cancer cells. In present work we offer the new method to detect in first morphological signs of cancer cell membrane (morphomarkers), which detects precancerous lesions for treatment and second- to specify spectral properties of morphomarkers by using Synchrotron Based IR Microspectroscopy (SB FTIRM).

Results: A technique for revealing surface morphology of human cervical cancer cells has been developed to facilitate early diagnostics of a pre-cancer and cancer cells under reflected light microscopy. To measure spectral features of morphological markers of cervical cancer cells (so named disperse lightened particles (DLP)), we used Synchrotron Based IR Microspectroscopy (SB FTIRM) in the mid-IR range (2 to 25 mkm wavelength). We used point-by-point IR microspectroscopy analysis in confocal geometry for high resolution for cervical cancer cells.

Conclusion: We discovered morphological features of cervical cancer cell membrane (morphological markers) for early diagnostics cervical cancer instead of widespread Pap-test. These morphological markers look like bubble is filled with products of cancer cell exocytosis. Application of the reflected optical microscopy let us to clearly visualize quite important morphological features of the malignized cells epithelial from uterine neck of patients with diagnosis *Cr. colli uteri* as aggregations of spherical multiple dispersed pathologic discharges on the cell surface with high reflectance. In summary, shows that in comparison of Pap-test offered the new method have next advantages:

Better sensitivity (about 100%).

- Two time cheaper in comparison with Pap-test Much more express.
- To test one sample need 5–10 min (Pap-test take 0.5 day)
- Easy-to-work.
- Two stage evaluate of sample. (Pap-test is many-stage treatment and evaluation (5–6 steps)).
- A little number of smears diagnostic signatures (3) against Pap-smear (8–10).

Disclosure of Interest: None declared.

O-077

Effect of Pregnancy on Postpartum Papanicolaou Test Results

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Objectives: Cervical cancer onset becomes youth with early first experience, and Papanicolaou tests performed in early pregnancy can detect cervical cancer. However, Papanicolaou tests during pregnancy have been noted to be inaccurate to reflect changes associated with pregnancy. Therefore, we assessed the effect of pregnancy on Papanicolaou test results.

Materials and Methods: Of 1351 pregnant women who delivered at Ise Red Cross Hospital between January 2010 and December 2014, 1213 underwent Papanicolaou tests at early pregnancy and postpartum. We compared the Papanicolaou test results of the women.

Results: The Papanicolaou test results in early pregnancy were negative for intraepithelial lesion or malignancy (NILM) in 1,191 patients, atypical squamous cells of undetermined significance (ASC-US) in 12, low-grade squamous intraepithelial lesion (LSIL) in 6, high-grade squamous intraepithelial lesion (HSIL) in 4, atyp-

ical squamous cells that cannot exclude HSIL (ASC-H) in 0. The Papanicolaou test results at postpartum were NILM in 1,187, ASC-US in 14, LSIL in 5, HSIL in 6, and ASC-H in 1. The results were different in 32 patients. Of the 1,191 patients with NILM in early pregnancy, 16 (1.3%) had other cytological abnormalities at postpartum. We performed therapeutic conization in four patients at postpartum. The Papanicolaou test results in early pregnancy of the four patients were NILM in 1 patient, ASC-US in 1, and HSIL in 2.

Conclusion: The usefulness of Papanicolaou tests during pregnancy can be overvalued or undervalued. Taking advantage of 1-month postpartum visits for screening with Papanicolaou tests could be useful to detect abnormal Papanicolaou test results, particularly false-negative ones in early pregnancy.

Disclosure of Interest: None declared.

O-078

Improvement of Conventional Pap Smear Adequacy and Accuracy in Women That Underwent Anti-Inflammatory Treatment

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Objectives: Conventional cytology is still the method of choice for screening of cervical carcinoma in many countries including Poland. Inflammatory conditions may limit the adequacy of smear and consequently lead to pitfalls in diagnoses. Cooperation of gynaecologists and pathologists is necessary to minimize false negative and false positive results. However, gynaecologists do not will to employ anti-inflammatory treatment in cases that are clinically silent and infective agent is not identified.

Material and Methods: We reviewed conventional smears from 581 women that had Pap test repeated after anti-inflammatory treatment in years 2013–2015. In all these patients the initial smears were inadequate for diagnosis due to intense inflammation. In 63 cases the infective agent was identified in initial smear.

Results: 93/581 (16%) smears were still inadequate for diagnosis due to intense inflammation. In 488 smears the adequacy was obtained for proper diagnosis. 471/488 smears (96.5%) were diagnosed as negative. 90/488 smears (18.44%) were within normal limits. 314/488 smears (64.34%) were adequate for diagnosis with benign reactive changes associated with inflammation. In 52 women (10.65%) presence of erythrocytes, cytolysis or low cellularity were observed. 17 smears in a group of 488 were diagnosed as positive (3.48%). ASCUS was diagnosed in 3 women, LSIL was diagnosed in 9 cases, ASC-H and HSIL were equally diagnosed each in 2 cases and in one smear squamous cell carcinoma was present.

Conclusion: Our results indicate the improvement of adequacy and accuracy of conventional cytology obtained after anti-inflammatory treatment. Occurrence of positive smears (including cancerous cells) suggests that employment of anti-inflammatory treatment followed by repeated cytology is highly reasonable.

Disclosure of Interest: None declared.

Respiratory Tract

O-079

The Thoracal Mass Profile by CT Scan Guided Fine Needle Aspiration Biopsy 2012–2013 in Dr. Soetomo Hospital Surabaya Indonesia

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Introduction: Many types of cancer including lung cancer cases increased recently in Dr. Soetomo Hospital Surabaya, Indonesia. Fine Needle Aspiration Cytology (FNAC) offers a certain advantages especially for late stage malignancies. Aim of this study is to provide the profile of Fine Needle Aspiration Cytology (FNAC) CT Scan guided result as a simple minimally invasive procedure with high advantages during period 2011–2013.

Material and Method: A retrospective and descriptive study was done, using secondary data of FNAC result in Dr. Soetomo Surabaya. The sample were FNAC specimens of the patients from Department of Pulmonology with clinical diagnoses as thoracal mass who underwent FNAC CT Scan guided in the Department of Radiology and were analiyzed in Pathology Laboratory Dr. Soetomo Hospital period January 2nd, 2012 until December 31st, 2013 then were classified mostly based on sex, age, location, cytological diagnoses and tumor stage.

Result: There were 437 patients with thoracal mass sent to the Department of Radiology, consist of 421 (96.34%) pulmonary lesions and 16 (3.66%) mediastinal lesions. There were 283 (67.22%) male and 138 (32.78%) female patients. Mostly affected to the 6th decade (33.73%), the youngest patient was 21 years old male and 27 years old female, the oldest was 87 years old male and 81 years old female. The most common site was the right lung 225 (53.44%) cases. The most cytological finding consist of primary lung cancer 261 (62%), metastatic lung cancer 26 (6.18%). The most of lung cancer was adenocarcinoma 179 (68.58%) and squamous cell carcinoma 74 (28.35%). Tuberculosis represented 9.98% cases and inconclusive result were 28 cases (6.65%).

Conclusion: FNAC CT Scan guided is useful for determining between pulmonary and mediastinal lesions that can produce a proper diagnostic in easier way comparing with surgical procedure especially for advance cases.

Disclosure of Interest: None declared.

O-080

Fine Needle Aspiration Cytology Diagnosis of Thoracal Mass CT Scan Guided in Dr. Soetomo Hospital, Surabaya Indonesia

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Background: Indonesia as developing country has a huge health problem that is following the global trend and cancer becomes complicated due to the increasing cases with low resource both human and facilities. Dr. Soetomo Hospital is the biggest hospital in Indonesia, has 1,550 beds capacity located in Surabaya East Java province, has developed and as pioneer in fine needle aspiration cytology diagnostic services. Within two years 2012 and 2013, the data shows totally 1,068 cases of thoracal mass both male and female patients which consist of 448 cases in 2012 and 620 cases in 2013, that almost 80% were advance malignancies. Fine Needle Aspiration Cytology has offered many advantages especially in shortening the response time to make a proper diagnoses that is needed for national referral hospital. CT Scan guiding FNAC of the thoracal mass can determine inflammation or infection, malignant lesions, clinical stage, presence of metastatic tumor, after treatment follow up and tumor recurrence which is very helpful for the patients and the doctors.

Objective: To obtain a simple procedure which can shorten the diagnostic procedures of thoracal mass management.

Method: Patients form Department of Pulmonology who got thoracal mass came to the Department of Radiology and puncture biopsy using 25 G spinal needle were done under guided of CT Scan in collaboration between the radiologist and pathologist.

Accuracy: Diagnostic accuracy of Aspiration Cytology in pulmonary neoplasm especially for metastatic tumor is very high, more than 90%, while in Dr. Soetomo Hospital has been investigated in 2012 and the result was 92.82%.

Result: Using Fine Needle Aspiration Biopsy CT guided there were 437 patients with thoracal mass and the result showed 421 patients with pulmonal lesions and 16 patients with mediastinal lesions. 62% among pulmonal mass were primary lung cancer, 6.17% were metastatic lung cancer and the rest 31.83% were benign lesions. Most of the patients were in T3 and 4 for 49.04%. Within 30 minutes the pathological result can be delivered and there was no complication.

Conclusion: Fine Needle Aspiration Cytology method under CT scan guiding is a minimally invasive procedure that is important in making diagnoses for thoracal mass because it is a simple technique which is relatively easy and very useful to shorten the response time that can improve the hospital service and the economical reason is fit for developing country like Indonesia.

Disclosure of Interest: None declared.

O-081

Cell Block of Pulmonary Cytology Sample Could Be Useful If Biopsy Was Negative

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Objective: In this study, we examined routine liquid-base cytology (LBC) data of the lower respiratory tract and lung to evaluate significance of LBC in pulmonary cytology and cytopathology.

Materials and Methods: Samples: Among 570 cases including trans-bronchial cytology (TBC) and lavage fluid of fine needle aspiration biopsy (LB-FNAB) operated in Nakagami hospital from July of 2012 to October of 2013, clinically lung cancer was suspected 415 cases were examined based on the permission of ethical review board of both Nakagami Hospital and University of the Ryukyus, School of Medicine. The biopsy samples submitted on the same date as of cytology were also reviewed.

Evaluation: For cytological and histological evaluation, scores was given to each specimen as followings; negative for atypia or no atypia; 1, atypia due to reactive change; 2, benign tumor or atypical cells unable to exclude possibility of malignancy; 3, suspicious of malignancy; 4, and malignancy; 5. Independently judged scores by 2 pathologists were used for this study. Three differently scored cases were eliminated from this study.

Results: Because LB-FNAB cases was small (n = 22), we analyzed TBC cases. Simultaneously collected brushing and washing samples were analyzed. Percentage of score 5 was higher in brushing cases than washing cases (37.50% vs. 25.54%). In 60 cases of which CB samples were available in both brushing and washing samples was analyzed. Scores of brushing of LBC sample and the ones of CB-brushing sample were significantly higher than the ones of washing of LBC sample or CB-washing sample ($p = 0.005$, and $p = 0.040$ respectively). Because biopsy score contained less score 2, we compared the score of LBC and biopsy by 3 grades (score1+2, score 3, and score 4+5). Between biopsy score and LBC score, there was no statistical difference. Also there was no statistical significance between CB score and biopsy. However, if higher score was selected from brushing and washing samples and the score was compared with biopsy score, score was higher in CB samples, and tendency of difference was observed ($p = 0.059$).

Conclusions: Our data clearly showed that brushing was significantly highly scored than washing. In addition, although either CB-brushing or CB-washing alone has possibility to negatively diagnose the cases, combination of brushing and washing of CB samples could complement biopsy.

Disclosure of Interest: None declared.

O-082

H-Score on Cell Blocks and Core Biopsies in Evaluating Epidermal Growth Factor Receptor (EGFR) Expression for Predicting a Response to Tyrosine Kinase Inhibitors in Non Small Cell Lung Cancer (NSCLC)

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Introduction: Lung cancer is a leading cause of death in India and worldwide, as patients present at a late stage, with a locally advanced and inoperable tumors. This emphasizes the need for a precise diagnosis by cytology with molecular studies to facilitate the choice of adjuvant therapy. NSCLC accounts for approximately 85% of all lung cancer and EGFR, a member of ErbB family of transmembrane tyrosine kinase receptors is one of the major oncogene involved. Overexpression of EGFR is associated with advanced stages of lung cancer and serves as an ideal target for therapy.

Objective: Significance of scoring systems (regular and H score) in evaluating EGFR expression in NSCLC and evaluation of EGFR expression as a prognostic factor to initiate anti EGFR therapy.

Material: Immunohistochemical (IHC) staining of EGFR expression of 50 cases of NSCLC diagnosed on cell block and core biopsies were evaluated both by regular and H scoring systems to note their significance in predicting a response in patients followed up for 6 months – with and without chemotherapy with anti EGFR drug.

Results: Of the total cases 54% were adenocarcinoma, 28% were squamous cell carcinoma, 14% were poorly differentiated carcinoma and the remaining 4% were of large cell carcinoma. Regular membrane scoring of EGFR showed 68% of cases to be positive (>Faint/partial membrane staining in >l 0% of tumor cells). 62% of EGFR positive cases were treated with carboplatin, paclitaxel and geftinib of whom 71% survived while only 23% of EGFR +ve patients without chemotherapy and 62% of EGFR -ve patients survived. On re-evaluating the EGFR staining by H Score, which considers both the different intensities of staining and percentage of cells stained with different intensities, the survival with chemotherapy was found to be 88% in patients with H score positivity (discriminatory threshold of 200). Hence, importance of H scores in evaluating EGFR expression in predicting a better response to tyrosine kinase inhibitors in NSCLC.

Conclusion: H Score shows better significance in evaluating EGFR expression in predicting a response to tyrosine kinase inhibitors in NSCLC.

Disclosure of Interest: None declared.

O-083

KDM4A, KDM4B and KDM4D in Non Small Cell Lung Cancer

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Objectives: KDM4A, KDM4B and KDM4D belong to jumonji group of KDM lysine demethylases demethylating H3 at lysine K9 and K36 sites, additionally KDM4D also demethylating the H1.4 linker histone at K26 lysine. We analysed their expression in lung tumors.

Materials and Methods: 188 lung tumors were analysed for the immunohistochemical expression of KDM4A, KDM4B and KDM4D. Additionally, the trimethylated state of chromatin was detected with an antibody H3. There were 132 squamous cell carcinomas, 53 adenocarcinomas and 3 large cell carcinomas in the study.

Result: There were significantly more cases with nuclear KDM4A expression in squamous cell carcinomas compared to adenocarcinomas ($p = 0.019$). Nuclear KDM4A and KDM4D was associated with the presence of lymph node metastases in tumors ($p = 0.049$, $p = 0.009$, respectively). Cytoplasmic KDM4A was associated with a worse survival of the patients ($p = 0.015$) and with a shorter recurrence free interval ($p = 0.028$).

Conclusion: KDM4A and KDM4D appear to have significance in the metastatic spread of lung carcinomas. The findings are in line with their proposed involvement in mechanisms associated with cell proliferation, apoptosis and DNA repair.

Disclosure of Interest: None declared.

O-084

Neuroendocrine Markers Possible Predictive Factors for Survival in Small Cell Lung Carcinoma

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Introduction: Small cell carcinoma comprises ~15–20% of lung carcinomas and usually presents with a hilar mass and disseminated disease. It has a poor prognosis, and most patients are not surgical candidates, so patients are treated with chemotherapy and/or radiation. Few markers are available for prognostication of outcome.

Methods: From 2008 to 2010, 136 cases of small cell lung cancer were evaluated for synaptophisin and chromogranin expressivity. Expression was categorized as positive or negative. The time to death from diagnosis was calculated for each category.

Results: Out of these specimens, it was determined that when synaptophysin, and chromogranin were each individually posi-

tive, the mean time to death from diagnosis was 352 days (mean age 64.6 years, N = 118) and 370 days (mean age 61.7 years, N = 66), respectively. When synaptophysin and chromogranin were negative, the mean time to death from diagnosis was 303 days (mean age 65.8 years, N = 14) and 284 days (mean age 64.9 years, N = 41), respectively. But if synaptophysin and chromogranin were both negative, the mean time to death from diagnosis decreased further to 272 days (mean age 64.3 years, N = 12).

Conclusion: Loss of synaptophysin and chromogranin expressivity did not significantly alter the mean age at which small cell lung cancer presents. However, loss of these immunomarkers portended a shorter survival time for the patients involved.

Disclosure of Interest: None declared.

O-085

Functionalized (C60) Fullerene Causes Oxidative Stress in Human Embryonic Lung Fibroblasts by Neutralizing Reactive Oxygen Species

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Objectives: It is a matter of common knowledge that [C60] fullerenes and their derivatives have antioxidant properties due to their chemical structure. Studies show that these substances also possess anti-viral and anti-cancer activity, moreover, they are under studies as potential nanocarriers for drug delivery across biological barriers. But the effect of such derivatives on human cells is still controversially discussed and needs further investigation. Here we assessed the time-dependent effect of a water soluble fullerene derivative that contained five residues of 3-phenylpropionic acid and a chlorine substituent attached to the carbon cage (F-828) on reactive oxygen species (ROS) production, NOX4 level and oxidative stress in human embryonic lung fibroblasts (HELFs).

Materials and Methods: Various concentrations of F-828 were added to HELFs at 37°C in the DMEM medium. The mRNA levels were analyzed using the StepOne Plus (Applied Biosystems). Protein levels were analyzed using CyFlow Space (Partec, Germany). Fullerene localization was assessed by means of fluorescent spectroscopy. The levels of the ROS were evaluated using H_2DCFH-DA assay.

Results: During the first 24 hours of incubation with HELFs F-828 localizes on the surface of the cells and in the medium and doesn't penetrate into the cytoplasm. 15 minutes after start of incubation with F-828 (in concentrations 0.004, 0.1, 4 and 19 μM) the amount of ROS on the cell surface drastically reduces thus leading to the activation of *NOX4* transcription. Increased level of NOX4 leads to the increment in ROS production. Three hours later both NOX4 and ROS level deflates (early response). 24 hours after addition F-828 penetrates into the cytoplasm and neutralizes matrix ROS causing secondary peak in NOX4 synthesis and ROS production and thus oxidative stress (late response). Both ROS reduction and oxidative stress were proportional to the concentrations added. Investigated fullerene derivative doesn't penetrate into cell nuclei, however it affects ROS generation there.

Conclusion: A new mechanism of ROS regulation by fullerene derivatives in human cells was studied. Fullerene derivative F-828 vigorously neutralizes ROS. This excessive anti-oxidant activity causes oxidative stress by binding all ROS. However, brief contact with F-828 lowers ROS amount in the medium and on cell surfaces, thus, it can potentially be used in situations when a rapid ROS neutralization is needed, for example after exposure to ionizing radiation.

Disclosure of Interest: None declared.

O-086

Reexamination of the Negative Cytology of the Respiratory Tract

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Background: Cytological specimens of the respiratory tract are checked by a cytotechnologist, and if positive or suspicious cells are observed, also by a cytopathologist. However, most of the negative specimens are checked only by a single cytotechnologist. To examine the accuracy of the determination of negative for specimens, cytological specimens obtained from fiberoptic bronchoscopy were reexamined. Fiber optic bronchoscopy was performed in 110 patients. Among them, 80 patients were diagnosed as primary lung cancer by transbronchial lung biopsy. Transbronchial biopsy was followed by brushing and washing cytology. In total, 154 cytological specimens were obtained from the same patients, of which 24 (10 brushing and 14 washing) specimens determined as negative were reexamined.

Results: Atypical cells that were hard to distinguish between columnar cells and adenocarcinoma cells were observed in one of the 24 specimens. Determination of the histological type was also difficult based on biopsy specimens of this patient. Obvious cancer cells were not confirmed in the other specimens. Only a few epithelial cells were observed in most of the negative specimens of washing cytology, due to a large number of red blood cells.

Conclusion: Negative predictive value of the brushing and washing cytology was high. So as not to disadvantage patients, regular check of negative specimens is necessary. The sample size should be prescribed depending on the ability of the institution.

Disclosure of Interest: None declared.

O-087

Correlations between the Results of the Histological Examination and Cytological Features in the Diagnostic of the Small Peripheral Lung Tumors

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Objectives: With the development of high-resolution computed tomography (CT), small-sized tumors showing ground-glass opacity (GGO) on chest CT images has been more frequently encountered. There have been some reports on transbronchial biopsy (TBB) through endobronchial ultrasonography with a guide sheath (EBUS-GS) for diagnostic sampling of GGOs. However, technique such as EBUS-GS is limited in their ability to diagnose such small lung tumors. There have been various studies for the cytological characteristics of small peripheral adenocarcinoma to assess the accuracy but with variable results. The discussion about the cytological features of small tumors with GGO in detail is necessary. We evaluated about the association of the cytological features with the histological examination using the surgically resected specimen. 75 patients, age between 23–83 years old, who showed clinical and radiological signs of peripheral lung tumors less than 3.0 cm in diameter, underwent surgical resection at our institution between 2013 and 2014.

Material and Methods: Imprints or touch preparation were preparad from surgical specimen. Papanicolaou's stain were employed in all cases. Tissue fragments taken from surgical specimen were fixed with 10% neutral buffered formalin and stained with hematoxylin and eosin.

Results: By histological examination (in the 75 cases), the diagnostic of lung cancer was given with the establishing of the histological type. In 38 cases (77.3%) of the cases diagnosed as adenocarcinoma, in 11 cases (14.7%) squamous cell carcinoma, in 3 cases (4%) was neuroendocrine tumors, and three cases were other histological type. In the 75 cytological examined cases, 47 patients (62.6%) diagnose of lung tumors are below 2.0 cm in size. In 32 of the 47 cases (82.6%), the result of cytological examination was adenocarcinoma. Tumor stamps of small sized adenocarcinoma are characterized by moderate cellularity and are composed of atypical cells arranged in small flat sheets. The nuclei are generally round, slightly hyperchromatic with small nucleoli.

Conclusions: Our data indicate the fact that the cytological examination on stamps from surgical material offers a very high percentage of positive results. But in the tumor size less than 1.0 cm, the establishing of the histological type of lung cancer is more difficult by cytological examination. Despite this, the cytology may be extremely useful in diagnose of the small peripheral tumors.

Disclosure of Interest: None declared.

Soft Tissues and Salivary Gland

O-088

Feasibility of Expression of HPV16, 18 and p16 as Biomarker for Distinguishing Normal Oral Mucosa from Oral Epithelial Dysplasia and Oral Intraepithelial Neoplasia

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Objectives: Numerous criteria exist for the diagnosis of oral epithelial dysplasia (OED), and there is not always a clear-cut distinction criteria between what represents mild dysplasia consisting only focal atypia, moderate dysplasia, and severe dysplasia which may present carcinoma in situ (CIS). According to the general rules for clinical and pathological studies on oral cancer, though mild and moderate dysplasia is defined as OED and sever dysplasia is done as oral intraepithelial neoplasia (OIN). As for CIS or OIN, however, a definitive distinction cannot always be drawn between mild and moderate dysplasia and CIS/OIN. Though OED and CIS are defined by dysplastic cells in the epithelium, accurate clinical as well as histopathological diagnoses has been contraversial. Specific markers are then needed to distinguish them. Furthermore, correct diagnosis and timely treatment of PMDs may help prevent malignant transformation in OIN. This preliminary study was planned to evaluate the expressions of human papillomavirus (HPV16, 18) and p16 would be feasible biomarkers to distinguish normal oral epithelium (NOE) from OED and OIN.

Materials and Methods: Subjects comprised 83 patients including 41 with OED (20 men; 21 women, mean age: 65.2 years), 30 with OIN (12 men; 18 women, mean age: 71.7 years) and 12 healthy controls (5 men; 7 women, mean age: 60.7 years). All patients underwent preoperative biopsy and/or smear cytology at our department. HPV 16 and 18 expressions in the formalin fixed and paraffin embedded and/or smear specimens were determined by immunohistochemistry using Anti-HPV16 E1+E4 antibody (abcam, Cambridge, UK, diluted at 1:100) and Anti-HPV18 E6 antibody (abcam, Cambridge, UK, diluted at 1:500). As a surrogate marker of HPV presence, $p16^{INK4a}$ (VENTANA, Arizona, USA, ready to use) was also used. Labeling indices (LIs) of HPV16, 18 and p16 were then examined. Regression tree analysis (RTA) was performed to distinguish NOE, OED/OIN. The independent variables included were p16, HPV16, HPV18, and the combined results of age. To predict OED/OIN, the primary variable for hierarchical tree was the combined results of p16, and the secondary variable was age (60 years), and the tertiary was HPV18.

Result: Statistically significant difference was seen in the expression of HPV18 and p16 of OED/OIN compare to NOE ($p < 0.001$). In all the cases of OED/OIN, p16 showed positive. Cases with p16 negative, age >60 years and HPV18 positive showed 90% of OED/OIN, those with p16 negative, age >60 years and HPV18 negative showed 60% of OED/OIN, and p16 negative, age ≤60 years a showed 30% of OED/OIN.

Conclusion: The expression of p16 and HPV18 and patients' age (60 years) would be feasible biomarkers to distinguish NOE and OED/OIN.

Disclosure of Interest: None declared.

O-089

Diagnostic Performance of Oral Cancer Cytology in a Pilot Study

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Objective: Exfoliative cytology is a reliable tool for assessing malignant change in various organs and recently, cytology has been applied to the diagnosis of oral lesions. Accurate cytological diagnosis of oral lesions, especially in distinguishing benign lesions from malignant ones, is essential for treatment as well as for clinical and epidemiological research including prognosis. The aim of this study is to explore diagnostic performance of oral cytology based on histological diagnosis as 'gold standard'.

Materials and Method: A total of 423 samples of oral cytology with accompanying histological slides provided from the members of Oral Cytology Working Group were screened. Histological diagnoses were classified as 'Negative', 'Borderline lesion -', 'Borderline lesion +', 'oral intraepithelial neoplasia/carcinoma in situ (OIN/CIS)', and 'Positive'. Cytological diagnoses were done according to the classification proposed by the Diagnostic Guideline Committee for Oral Cytology of Japanese Sociaty of Clinical Cytology and were classified into NILM (negative for intraepithelial lesion or malignancy), LSIL (low-grade squamous intraepithelial lesion), HSIL (high-grade squamous intraepithelial lesion), SCC (squamous cell carcinoma), Other malig (other malignancy) and IFN (indefinite for neoplasia or non-neoplasia). These slides for cytology were evaluated by 10 raters. The cytology results were compared with histology results. To compare between cytological and histological diagnosis, the histological diagnosis were classified into 'Negative' (Negative' and 'Borderline lesion -') and 'Positive' ('Borderline lesion +', OIN/CIS' and 'Positive')[N1], and cytological diagnosis were also classified into 'Negative' (NILM and LSIL) and 'Positive' (HSIL, SCC, Other malignancy and IFN). Protocol for this study was approved by Ethics Committee at Shimane University Faculty of Medicine (Approval no.1270; March 29, 2013).

Result: Among 423 samples, 96 samples were excluded from the study because of their poor quality. Finally, among the remaining 327 samples, 285 samples reviewed. The results of cytological diagnoses for 5 cytological slides were not agreed by raters. In all cases, 2 or 4 raters diagnosed as NILM whereas the others diagnosed as HSIL. In 4 cases, histological diagnoses was 'Positive' (squamous cell carcinoma, moderately differentiated), while another 1 case was 'Negative' (epithelial dysplasia, mild). The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were 67.7%, 83.8%, 79.3% and 74.0% in case of cytological 'Negative' was NILM and LSIL, and 87.7%, 50.5%, 61.8% and 81.8% in case of cytological 'Positive' is LSIL, HSIL, SCC, respectively [N2].

Conclusion: Oral cytology would be applicable as one of the diagnostic tools of oral lesions.

Disclosure of Interest: None declared.

O-090

Agreement between Rapid On-Site Evaluation and Final Cytologic Diagnosis of Salivary Gland Specimens

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Objective: Rapid on-site evaluation (ROSE) has been shown to be beneficial in determining adequacy and preliminary diagnosis. Numerous studies to date have shown improvement of adequacy with ROSE; however, little is known regarding the diagnostic value and agreement of ROSE compared with the final cytologic diagnosis in salivary gland specimens. Our aim was to evaluate diagnostic agreement and compare performance (accuracy) between ROSE and the final cytologic diagnosis of salivary gland specimens, using the final histopathologic diagnosis as the gold standard.

Materials and Methods: All patients with salivary gland lesions who underwent fine-needle aspiration (FNA) with ROSE during January 2009 to December 2013 were evaluated. The patient demographic data, clinical characteristics, ROSE, final cytologic diagnosis and final histopathologic diagnosis were obtained. The cytologic diagnosis was categorized into six groups: benign, atypical, suspicious for malignancy, malignant, indeterminate and inadequate. Agreement and performance were assessed by Kappa statistic and receiver operating characteristic (ROC) curve analysis, respectively.

Results: A total of 347 patients (median age 52 years, 59% female) underwent FNA with ROSE of 386 lesions including parotid glands (64.2%), submandibular glands (29.5%), minor salivary glands (1.8%) and unknown (4.4%). On follow-up, 171 (44.3%) had the histopathologic diagnosis, 134 (34.7%) had clinical follow-up and 81 (21%) were lost to follow-up. Agreement between on-site and the final cytologic diagnosis was good to excellent (simple kappa = 76% [95% CI, 0.68–0.84]; weighted kappa = 81% [95% CI, 0.73–0.89]). The on-site interpretation was changed in the final cytologic diagnosis in 26 lesions (7.1%). Only one out of 36 malignant lesions reported by ROSE was downgraded to suspicious. Six initially reported benign lesions were upgraded to suspicious or malignant. The final cytologic interpretation yielded higher sensitivities (75% vs. 69.23%) and similar specificities (91.59% vs. 93.46%); however, no significant difference was found in performance between ROSE and the final cytologic diagnosis (area under the ROC curve = 82.39% vs. 84.54%, p = 0.826).

Conclusions: There was excellent agreement and comparably good performance between rapid on-site and the final cytologic evaluation in detecting malignant lesions of salivary glands.

Disclosure of Interest: None declared.

O-091

Application of the Adjuvant Molecular Methods for Minimally Invasive FNA Diagnostics of Lipomatous Soft Tissue Tumors

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Aims: Lipomatous soft tissue tumors comprise the most common category of primary soft tissue neoplasias, presenting with a wide range of clinical manifestations and aggressivity. Only the correct tissue diagnosis allows the proper management of the patients, according to the subtype of the tumor. We analyzed the combined value of the minimally invasive cytologic diagnostics with the clinical presentation, imaging and molecular auxiliary methods for diagnosing and precise subtyping of the lipomatous soft tissue tumors according to the current WHO classification.

Methods: Retrospective analysis of the archive data over 11 years (2005–2015) was performed. Fine needle biopsies (FNBs) were performed both in the walk-in-clinics of the Division of Cytology of the University Hospital Zürich, as well as in external facilities under various imaging guidance methods. Alcohol fixed, Papanicoloau stained, direct smears and paraffin embedded cell blocks (thrombin method) were prepared for all the FNBs. The genetic properties of the tumor tissue were studied, where indicated with FISH (*mdm2*, *DDTI3*, *FUS* and *EWSR1* genes), and/or RT-PCR/sequencing.

Results: Between 2005 and 2015, 427 tumors were diagnosed as lipomatous neoplasias in the Cytology Division of the Institute of Surgical Pathology of the University Hospital in Zürich. There were 396 benign lipomas (incl. variants) and 31 liposarcomas (LPS): 17 myxoid/round cell (MRLPS), 11 well differentiated (WDLPS)/dedifferentiated and 3 pleomorphic. In 43 cases – upon the consideration of the clinical presentation and imaging – molecular testing was applied (38 FISH, 15 RT-PCR, partially concomitantly). The correlation with the histology showed discrepancies in 3 cases: in two cases the *mdm2* gene FISH was not performed on the FNB sample, leading to the diagnosis of benign lipoma instead of a WDLPS/atypical lipomatous tumor, and in one case the RT-PCR reaction yielded falsely negative result in a case of a myxoid LPS.

Conclusions: Cytological samples of the fine needle biopsies are well suitable for the final diagnostics of the lipomatous soft tissue neoplasias. The necessary requirements for successful final tumor subtyping are: careful correlation with the clinical presentation and imagining, as well as knowledge of the differential diagnostic aspects according to the valid WHO classification.

Disclosure of Interest: None declared.

Thyroid 2

O-092

The Correlation of Thyroid Gland Bethesda System with Clinical Background, FNA Indications and US Findings

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Objective: The background clinical data of patients undergoing thyroid FNA have been scarcely analyzed in the relation to the Bethesda system. We analyzed clinical settings, indications, findings and consequences of the thyroid gland FNAs within one-year-period in Pirkanmaa District area (population of 0.52 millions).

Materials and Methods: Retrospective series consisted of 415 thyroid FNAs from 363 patients. The female-to-male ratio was 4:1. The median age was 59 years. The clinical data including FNA indications, clinical signs, laboratory and US data were collected. If the management after FNA was surgical, final histopathological diagnosis was also revised.

Results: Two thirds of FNAs were taken in primary healthcare. Only in 48%, the patient was euthyroid. Nodule or nodular goiter was indication for US in 61%. Indication for FNA was nodule in 85%. The Bethesda system categories were represented as follows: non-diagnostic in 94 cases (26%), benign in 177 cases (49%), AUS/FLUS in 32 cases (9%), follicular neoplasm in 31 cases (9%), suspicious for malignancy in 20 cases (6%) and malignant in 9 cases (3%), respectively. New FNA was performed in 51 cases and thyroid surgery in 67 cases (22%). Final histopathological diagnosis was malignant in 27 cases including one non-diagnostic and one AUS/FLUS case on FNA.

Conclusions: The sensitivity of the Bethesda system was 81%, the specificity 88%, PPV 79% and NPV 90% when follicular neoplasia was excluded.

Disclosure of Interest: None declared.

O-093

Performance of the Different Classification Schemes of the Thyroid FNAs in Two Centers

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Objectives: As the imaging methods refine, the number of incidentally discovered thyroid nodules increases. The management of patients with both, clinically detectable and incidental nodules strongly depends on their estimated malignant potential. Fine needle aspiration (FNA) is the most important study in the examina-

tion and triaging of the thyroid nodules, providing essential information on the content of the intrathyroidal masses. The recently introduced classification schemes (British Thy- and American Bethesda-systems) of the results of the FNAs of the thyroid nodules facilitate the communication between the cytopathologists and clinicians. The overall performance of the two different systems in various clinical settings may lead to different pathways of patient management.

Materials and Methods: The archives of two large academic centers in Switzerland using different classification schemes for reporting on the results of the thyroid FNAs were searched. All FNAs over a two year period (2012 and 2013) were analyzed in respect to the relative frequency of the diagnoses and the subsequent correlation to the histopathological diagnoses of the resected nodules.

Results: In the center A using the modified British system 6% of the samples were non-diagnostic (Thy1), 7% were cystic without thyreocytes (Thy1c), 74% were classified as benign (Thy2), 7% as undetermined (Thy3) and 7% as either highly suspicious (Thy4) or definitely malignant (Thy5) The histological follow up was available for 17% of cases. The sensitivity and specificity was 100% and 98.6% respectively. In the center B applying the US Bethesda system the rate of the non-diagnostic FNAs was 26.6% (BI), 3.5% were cystic without thyreocytes (BI, cystic fluid only), benign 61% (BII), undetermined 6.1% (4.1% BIII and 2% BIV), 2.8% highly suspicious (2.0% BV) or definitely malignant (0.8% B VI). In center A as many as 74% of the FNAs of the thyroid nodules are perfomed by the cytopathologists, while none of the FNAs are done by the cytopathologists in the center B.

Conclusions: The performance of the classification schemes of the thyroid FNAs varies among centers due to inherent differences in the different reporting systems and to the differences of local factors in sample quality, related to the collection issues.

Disclosure of Interest: None declared.

O-094

Comparison between Conventional and Bethesda System for Reporting Thyroid Cytopathology: A Study from Indian Metropolitan City

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Objective:

1. To Compare Conventional (CSTC) & Bethesda system of reporting thyroid cytopathology (TBSRTC)

2. To determine sensitivity (SN), specificity (SP) & false positive rates for both methods

3. To correlate cytological diagnosis with histopathology (HPE) wherever available

Material and Methods: 2647 cases presented with thyroid swelling from 2010–15. FNAC was performed (standard technique). 2–3 smears were stained & rest slides were kept for immunocytochemistry (ICC). Smears from all cases were reported by CSTC & TBSRTC. CSTC at our centre includes 13 categories:

Inadequate, Adenomatoid Goitre (AGT), Colloid Goitre (CGT), Acute Thyroiditis (AT), Subacute Thyroiditis (SAT), Lymphocytic Thyroiditis (LT), Granulomatous Thyroiditis (GT), Hashimotos thyroiditis (HT), Follicular Neoplasm (FN), PapillaryCarcinoma (PCT), Medullary Carcinoma (MCT), Anaplastic cell Carcinoma (ACT), Other Malignancy (OM).

Categorization for TBSRTC done using criterias in atlas.

Statistical parameters were calculated with cytology as screening test & HPE as gold standard.

Results: 238 (8.9%) were inadequate both by CSTC & TBSRTC.

Remaining 2409 cases: CSTC-2270 (85.7%) cases were benign [AGT (170), CGT (1544), AT (9), SAT (4), LT (204), GT (39), HT (300)].

139 (5.2%) cases were malignant [FN (43), PCT (60), MCT (23) & OM (13)].

TBSRTC-2254 (85.1%) cases were benign [CGT/AGT (1551), AT (160), LT/HT (497), GT/others (46)], 15 (0.5%) were atypia of undetermined significance (AUS), 47 cases (1.7%) were follicular neoplasm/suspicious of follicular neoplasm (FN/SFN), 42 (1.5%) were suspicious of malignancy (SM) with PCT (30), MCT (5), Lymphoma (5), ACT (2). 51 (1.9%) cases of definite malignancy (DM) with PCT (30), MCT (18), ACT (1) & other (2).

HPE in 278 cases.

Out of 14 cases diagnosed unsatisfactory on FNAC; 2 cases were malignant on HPE with PCT (1)& FCT (1).

Out of 2239 benign cases, 150 were benign on HPE.

Out of 15 cases of AUS, 12 received; malignant (10)-4 cases of Follicular variant of PCT (FVPCT) & FCT (6) [All categorized as benign AGT/CGT by CSTC] & benign (2).

Out of 47 cases of SFN, 35 were received-follicular adenoma (12), FCT (19), FVPCT (4).

Out of 42 cases of SM, 30 were received with PCT (15), MCT (7), NHL (2), ACT (1) & others (5).

Out of 51 cases of DM, 32 were received with PCT (20) & MCT (12).

CSTC had SN (88.81%), SP (99.47%), Positive predictive value (PPV) of 91.36% and Negative predictive value (NPV) of 99.29%. TBSRTC had SN (100%), SP (99.38%) (including FN/SFN category), SP (100%) (excluding FN/SFN category), PPV of 91.08% (including FN/SFN category), PPV of 98.62% (excluding FN/SFN category) & NPV of 100%.

Conclusion: TBSRTC effectively seggregates definite malignant lesions from benign ones at the same time maintaining high index of suspicion for cases with subtle atypia. This ensures high sensitivity and specificity giving it a clear edge over CSTC.

Disclosure of Interest: None declared.

O-095

Loss of Cellular Polarity/Cohesiveness in Papillary Thyroid Carcinoma, a Precursor Lesion of Hobnail Features? Based on a Case Report and Review of the Literature

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Objective: Loss of cellular polarity/cohesiveness and hobnail features are newly described morphological features in papillary thyroid carcinoma. Immunohistochemical and molecular feature of loss of cellular polarity/cohesiveness, hobnail features and micro-papillae were examined and the correlations between these features were determined.

Material and Methods: A case with gradually increased proportion of LOP/C and hobnail features in his twice recurrent tumor was evaluated using immunohistochemical staining. $BRAF^{V600E}$ mutation and RET/PTC 1 rearrangement was analyzed using DNA sequencing.

Results: A 58-year-old man suffered from papillary thyroid carcinoma with twice recurrence was presented. Tumor cells with LOP/C and hobnail features were increased gradually in twice recurrences and marked micro-papillae were presented in the cell block of the second recurrence. Immunohistochemical staining showed positive immunoreaction with cytokeratin 19, HBME-1 and Galectin-3. Decreased and none immunoreaction with TTF-1 and increased immunoreaction with ki-67 index were identified respectively in the first and second recurrence. Gradually decreased immunoreaction with E-cadherin, ß-catenin and gradually increased immunoreaction with vimentin were determined by immunohistochemical staining. Lumen-side lining type and inside-out immunoreaction with EMA was found in the primary tumor, the first and second recurrence, respectively. $BRAF^{V600E}$ mutation was detected, however, RET/PTC-1rearrangement was absent.

Conclusion: Dedifferentiation was proved in the twice recurrence of the present case according to the immunoreaction with TTF-1 and ki-67. Loss of immunoreaction with epithelial makers and gain of the immunoreaction with mesenchymal marker were observed, which indicated that epithelial mesenchymal transition occurred at the same time with the dedifferentiation. The gradual immunoreaction with all the markers indicated that LOP/C is probably the precursor lesion of hobnail feature and micro-papillae in papillary thyroid carcinoma.

Disclosure of Interest: None declared.

O-096

Should Cyst-Fluid-Only Cases Be Classified into Nondiagnostic or Unsatisfactory Category?

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Objectives: According to the Bethesda System for Reporting Thyroid Cytopathology (BSRTC), cyst-fluid-only (CFO) cases, which represent abundant hemosiderin-laden macrophages and cyst fluid contents, are classified into the category 'Nondiagnostic' or 'Unsatisfactory' (ND/UNS), because one cannot exclude a possibility of cystic papillary carcinoma. It has been reported that risks of malignancy for CFO and ND/UNS excluding CFO were 4% and 1% to 4%, respectively. In contrast, CFO has been traditionally classified into 'Benign' in Japan. To reassess a significance of CFO, we compared CFO with ND/UNS except for CFO.

Materials and Methods: 10036 thyroid nodules underwent fine needle aspiration using 22-gauge needle at Kuma Hospital in 2007. 1500 (14.9%) of them were classified into ND/UNS, based on the criteria of BSRTC. The nodules were followed for up to 7 years. The ND/UNS samples were divided into two groups, CFO (720 samples, 7.2%) and ND/UNS excluding CFO (780 samples, 7.8%). We compared the two groups, regarding 1) repeated aspiration, 2) resection, and 3) risk of malignancy.

Result: The rates of repeated aspiration of CFO and ND/UNS excluding CFO were 9.7% (70 nodules) and 24.4% (191 nodules), respectively. Repeated aspirations at less than three months interval were performed in 11 (15.7%) of 70 CFO nodules and 97 (50.8%) of 191 ND/UNS nodules excluding CFO. Repeating the FNA results in a diagnostic interpretation in 63 (90.0%) of 70 CFO nodules and 150 (78.5%) of 191 ND/UNS nodules excluding CFO. In five of 6 CFO cases with re-aspiration, a malignancy had been suspected by the ultrasonographic examination (US), and in the remaining one papillary carcinoma was suspected by the aspiration cytology performed in the previous hospital and US was undetermined significance in our hospital. 76 (10.5%) CFO nodules and 103 (13.2%) ND/UNS nodules excluding CFO were resected, and the incidences of malignancy were 17.1% and 38.8%, respectively. There were no false positive cases with re-aspirations at less than three months interval. A risk of malignancy in the nodules classified into CFO and ND/UNS excluding CFO were 1.8% and 5.1%, respectively.

Conclusion: We demonstrated that the risk of malignancy in CFO was 1.8%, which were lower than that in ND/UNS excluding CFO. The incidence was 0–3% in 'benign' recommended by BSRTC. Therefore, we insist that CFO should not be included in ND/UNS but be handled as separate category, 'CFO', and follow-up or repeated aspiration should be considered, depending upon the ultrasound findings. In addition, we showed that repeated aspirations at less than three months interval were not problematic.

Disclosure of Interest: None declared.

O-097

Cytological Differential Diagnoses between Chronic Thyroiditis and MALT Lymphoma in Thyroid LBC

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Objectives: Chronic thyroiditis with nodular formation may undergo aspiration cytology to distinguish from MALT lymphoma. A cytological distinction between nodular chronic thyroiditis (NCT) particularly associated with predominant lymphocytes and MALT lymphoma (ML) has been frequently troublesome. In addition, the morphology in LBC specimens (LBC-S) is not same to conventional ones. The aim of this report is to clarify the difference of cytological findings between NCT and ML in LBC-S.

Materials and Methods: We reviewed LBC-S of 13 NCT cases and 17 ML cases confirmed by histologic examination in our hospital. LBC samples were obtained from wash-out fluid of the aspiration needles, fixed with Cytorich-RED™ and prepared by SurePath™ method.

Result: In 82.4% (14 cases) of ML cases and in 77% (10 cases) of NCT, lymphocytes greater than 8 μm in diameter occupied more than one-fourth of all lymphocytes. Cases in which lymphocytes associated with degenerative nuclear changes (discontinuous nuclear membrane and degenerative chromatin pattern) occupied more than one-fourth of all lymphocytes were observed in 76.5% (13 cases) of ML cases, but not in NCT cases. Elongated naked nuclei and cleaved nuclei more frequently appeared in ML cases than NCT. The incidence (100%) of more than four clusters composed of oxyphilic cells in NCT cases was significantly higher than that in ML (35.3%).

Conclusion: The findings that both lymphocytes greater than 8 μm in diameter and lymphocytes associated with degenerative nuclear changes occupy more than one-fourth of all lymphocytes are diagnostic clues indicating ML. The elongated nuclei, cleaved nuclei or no oxyphilic cell nests may be helpful in identifying ML from NCT on LBC-S.

Disclosure of Interest: None declared.

O-098

Analysis of Misinterpreted Hashimoto's Thyroiditis on Fine Needle Aspirations: A Cytohistologic Review of 16 Cases

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Objectives: Follicular epithelium in Hashimoto's thyroiditis may display a range of nuclear changes that mimic those encountered in PTC. We demonstrated cases which were misinterpreted aiming to identify cytological features observed in Hashimoto's thyroiditis leading to a misinterpretation and explore how to avoid an over diagnosis of malignancy.

Materials and Methods: Of a total of 2,312 FNA samples between March 2011 and May 2014 in our institution, 16 thyroid aspirates classified as suspicious for PTC or PTC cytologically while subsequently histology-proven Hashimoto's thyroiditis were reevaluated.

Results: 12 aspirates exhibited sparse or extremely few lymphocytes in the background which could be easily overlooked. One aspirate showed papillary-like tissue fragments with honeycomb architecture and two aspirates contained monolayered sheets with anastomosing trabeculae arrangement. Enlarged nuclei containing finely granular or powdery chromatin and micronucleoli presented in a few cell clusters of all 16 aspirates. Nuclear grooves were noted in 9 aspirates but most of them appeared thin and incomplete. No definite cytoplasmic inclusion was found. 7 aspirates contained sparse or a few multinucleated giant cells and only one aspirate showed several psammoma bodies. Lymphocytes infiltrating epithelial cell groups were detected in three aspirates. Markedly pleomorphic Hürthle cells with bizarre nuclei were observed in only one aspirate. The corresponding histological sections revealed counterpart of the cytological findings represented. The pitfalls included extremely sparse or not sampled lymphocytes, misinterpretation of papillary-like tissue fragments or monolayered sheets with honeycomb arrangement as syncytial tissue fragments. Over-emphasis placed on just one cytologic feature such as intranuclear grooves, powdery or watery chromatin, psammoma bodies or multinucleated giant cells, while other PTC-associated features were minimal or absent, will lead to a false positive diagnosis. A conclusion made based only upon sparse cell clusters, even typical neoplastic characteristic features confirmed within these clusters, is not reliable. We recommend the minimal criteria for the diagnosis of PTC, including syncytial tissue fragments, enlarged nuclei with powdery or watery chromatin and nuclear irregularity, nuclear pseudoinclusions, nuclear grooves and micronucleoli.

Conclusion: Extreme caution should be taken when rendering a positive diagnosis on the basis of a few areas of suspicion of the whole slide and no single cytopathologic feature is diagnostic as Hashimoto's thyroiditis may show several overlapping features with PTC. The minimal criteria for the diagnosis of PTC can help differentiate between the two and thus avoid a misinterpretation.

Disclosure of Interest: None declared.

O-099

Significance of Expert's Second Opinion on Thyroid FNA. Retrospective Study Based on Eight Years Material

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Objectives: The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) is based on six diagnostic categories: DC I = non-diagnostic, DC II = benign, DC III = atypia/follicular lesion

of undetermined significance (AUS/FLUS), DC IV = follicular neoplasm/suspicion for a follicular neoplasm (FN/SFN), DC V = suspicious for malignancy, and DC VI = malignant. This classification is in use since 2007. Second opinion given by experts in thyroid FNA evaluation is recommended especially in categories IV-VI.

Material and Methods: We reviewed thyroid FNA reports from one laboratory (Lublin) that were secondarily reviewed by experts in Thyroid FNA evaluation in main diagnostic centre in Poland (Gliwice), called referral centre. These FNA's were either of unusual cytological appearance, difficult for diagnosis or diagnosed in categories IV-VI. Histopathology was available in most of the cases confirmed or diagnosed as AUS/FLUS, FN/SFN, suspicious for malignancy and malignant. Cases of suspicious clinical or ultrasound presentation but classified as non-diagnostic or benign were also included.

Results: We reviewed the results of thyroid FNAs from 104 patients diagnosed in Lublin in years 2008–2015 that were also evaluated in referral centre. 9 smears treated as non-diagnostic (Lublin) were assessed as diagnostic (Gliwice) with 7 being malignant and confirmed by histopathology. 13 of diagnostic FNA's classified as benign were evaluated as malignant in referral centre (all confirmed by histopathology). 20 FNA's diagnosed as benign cytology in Lublin were confirmed benign. These benign smears were sent for second opinion due to suspicious clinical or radiological presentation. Statistical analysis showed that sensitivity of cytological diagnoses in Lublin was 72% and 92% in Gliwice (referral centre). Specificity was equal (100%). Diagnostic accuracy was higher for Gliwice (94%). In Lublin laboratory was 77%.

Conclusion: Unusual, difficult cytological cases that don't suit the standard diagnostic criteria, as well as cases diagnosed as FN/SFN, suspicious for malignancy and malignant, should be reviewed by pathologists experienced in thyroid FNA evaluation. Benign FNA's in patients suspicious on ultrasound or clinical examination should be also reviewed in referral centre before the FNA is repeated. This procedure improves the accuracy of cytological diagnosis.

Disclosure of Interest: None declared.

O-100

Thyroid Fine Needle Aspiration Cytology Mimicking Thyroid Papillary Cancer in a Lung Cancer Patient

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An enlarged malignant thyroid tumor can be primary or secondary. A patient of papillary thyroid carcinoma (PTC) with metastasis to the lung usually presents with a thyroid tumor and a lung nodule or nodules whereas a lung adenocarcinoma with metastasis to the thyroid can also present as a thyroid lesion along with a lung nodule or even nodules as a result of concomitant intrapulmonary metastasis. The modality of treatment and the prognosis between lung cancer and PTC are quite different. When a thyroid malignancy coexists with lung nodule(s), determination of the primary site is essential. The fine needle aspiration cytology (FNAC) features of PTC are characteristic but not pathognomonic. Moreover, a pulmonary adenocarcinoma especially papillary/micropapillary variant sometimes can show similar cytomorphologic features and confuse the diagnosis.

We would like to report a 58-year-old woman of lung cancer who was admitted because of left chest pain, headache, and shortness of breath on exertion for 2 weeks. Physical examination was unremarkable except for a grade II bilateral thyroid enlargement with firm to hard consistency and bilateral numerous small cervical lymphadenopathy. Chest X-ray (CXR) at physical check-up about 5 months in advance showed small nodules up to 9 mm in diameter in both lungs. Follow-up CXR at admission disclosed many small nodules but progressively increasing in size up to 13 mm in diameter in both lungs and bilateral interstitial infiltrates compatible with lymphangitic carcinomatosis. Her thyroid FNAC mimicked those of PTC. However, her clinical presentations are not in accordance with those of typical PTC. A preliminary right thyroidectomy showed features also resembling diffuse sclerosing variant of papillary carcinoma at frozen section. Immunohistochemically, however, the tumor cells were positive for thyroid transcription factor 1 (TTF-1) and napsin A but negative for thyroglobulin and paired box gene 8 (PAX8).

In conclusion, thyroid FNAC for PTC should be interpreted with caution in the presence of coexisting lung nodule(s). Clinical correlation may provide clues and warrant for further immunostains specific to thyroid origin. We report this patient so as to cause the awareness and attention during interpretation of thyroid FNAC in this scenario.

Disclosure of Interest: None declared.

Effusions

O-101

Interleukin-8 in Malignant Pleural Effusion Contributes to the Enhancement of Cancer Stem Cell Properties in Lung Cancer

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Objectives: Primary pulmonary adenocarcinoma causing malignant pleural effusion (MPE) usually indicates a late stage of cancer and initial metastasis. Previous studies have found that fluid in pleural cavity is rich in many kinds of growth factors and cytokines, especially interleukin-8 (IL-8), which is one of the most potential candidate mediators involving in carcinogenesis and progression in many cancers. Increased level of IL-8 in the tumor microenvironment has thought to be associated with tumor aggressiveness, enriched cancer stem cell (CSC) properties, and drug resistance. However, the underlying mechanism and the role

of IL-8 in the microenvironment of MPE that contribute to the tumor progression remain elusive.

Materials and Methods: We used NCI-H lung cancer cell line treated with IL-8 and cytology samples in patients with pulmonary adenocarcinoma and/or MPE for comparison focusing on the evaluation of the abilities of migration and invasion, the expression of CSC properties, and drug-resistance-related genes. Correlation of IL-8 in fluid cytology and cell block of MPE using immunocytochemistry with clinical parameters were also performed.

Results: Increased expression of IL-8 in the cell microenvironment enhances CSC growth, migration, invasion and drug resistance, promotes the sphere-forming ability, and also enriches CD133, Nanog, and OCT4. Furthermore, administration of IL-8 to lung cancer cell lines promoted cancer cell migration and invasion through induction of epithelial-to-mesenchymal trans-differentiation and increased CSC properties. IL-8 was not overexpressed in early lung cancer patients whereas overexpressed IL-8 was found in advanced, high-staged lung cancer patients with MPE. Furthermore, our results also demonstrated that IL-8-induced CSC properties were via IL-8/CXCR1 autocrinal signaling circuit. Expressions of IL-8 and CXCR1 in cell blocks correlated significantly with overall survival and co-expressions of IL-8 and CXCR1 were associated with poor prognosis.

Conclusion: Our study confirms that the platform of pleural effusions may provide a metastatic niche with plenty of a variety of cytokines. IL-8 and it receptor CXCR1 in MPE play a pivotal role in the fluid microenvironment and contribute to tumor progression in lung adenocarcinoma.

Disclosure of Interest: None declared.

O-102

Cytological Diagnosis of T Lymphoblastic Leukaemia/Lymphoma in Patients with Pleural Effusion: Report on Two Cases by an Algorithmic Approach Using Complete Immunohistochemical Phenotyping

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Background: T cell lymphoblastic leukaemia/lymphoma with an initial symptom of effusion is an uncommon event. It may present a diagnostic challenge as lymphoblastic cells mimic normal lymphocytes under low power microscopic view.

Materials and Methods: The first case was a 31 year old man, and the second case was a 54 year old man. Both of them initially presented with chest pain and shortness of breath. CT scans found an anterior mediastinal mass and left pleural effusion for both of them. Cytological smears of pleural fluid in both cases showed monotonous small to medium sized lymphoid cells with moderate chromatin condensation and round to convoluted nuclei. There were prominent apoptotic bodies and mitosis in both cases. Immunohistochemistry (IHC) of cell blocks demonstrated their T cell lineage and lymphoblastic nature. Diagnosis of T cell lymphoblastic leukaemia/lymphoma was accomplished by an algorithmic approach of three steps using complete panels of immunohistochemical markers (CD3, CD20, Ki-67, CD10, CD5, CD99, CD1a, CD34, CD4, CD8, TDT, CD7, CD2 and CD68 etc.).

Conclusion: An algorithmic approach based on cytological morphology and immunophenotyping is an effective way to diagnose T cell lymphoblastic leukaemia/lymphoma in patients with an initial symptom of pleural effusion and insidious cytological morphology.

Disclosure of Interest: None declared.

O-103

Cytomorphological Significance in Diagnosing Malignant Mesothelioma in Serous Effusions

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Objective: To discuss characteristic cytological features for the cytopathological diagnosis of malignant mesothelioma (MM) in serous effusion cytology.

Data Sources: Cytopathologists involved in the Japanese Society of Clinical Cytology (JSCC) and Jananese Lung Cancer Society (JLCS), and with an interest in the field contributed to this update.

Results: Recently, the cases of MM have been insidiously increased in number in not only Japan but also the foreign countries. We must grasp the morphologic characteristics of MM in serous effusions to diagnose MM and perform differential diagnosis of MM among adenocarcinomas and reactive mesothelia using by immunocytochemistry. Especially, mesothelioma markers, such as calretinin, D2-40, and WT-1, and adenocarcinoma markers, such as CEA, TTF-1, and CD15 etc. are useful in differential diagnosis between MM cells and adenocarcinoma cells. Moreover, EMA, desmin, GLUT-1, and CD146 etc. are useful in distinction of MM from reactive mesothelia. In Japan, the survey of effusion cytology of MM cases lead us to several cytologic characteristics.

Conclusion: 1) Background mucin-like material (hyaluronan), 2) collagenous stroma; Type 2 and Type 3, 3) 'hump'-like cytoprocesses, 4) increased frequency of multinuclear cells, 5) blurring cell margin of MM cells, 6) background pyknotic orangeophilic cells are useful features in ordinary specimens to estimate the diagnosis of MM, and these findings are statistically predominant.

Disclosure of Interest: None declared.

O-104

Rehydration before Wet Fixation in Conventional Body Fluid Cytology

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Objective: In conventional cytology, preparation of body fluid specimen such as pleural effusion and ascites by wet fixation for Papanicolaou stain on the slide glass potentially is subject to dry effect or cell loss if not processed properly. The disadvantages may make cytologic interpretation of the smear difficult or even impossible. We have been routinely making an additional smear for rehydration with normal saline before wet fixation so as to overcome the above potential shortcomings. We would like to report our experience in this method in conventional body fluid cytology.

Material and Method: We reviewed the result of body fluid specimen in our cytology laboratory over the past one year. Four slides of each specimen were made. Two were air-dried for Liu's stain (also known as Riu's stain, a Romanowsky stain) and the other two were wet-fixed by 95% alcohol for Papanicolaou stain. The air-dried smears were also served as cellularity control. One of the two wet-fixed smears was thrown into the alcohol jar for wet fixation immediately as soon as the specimen was smeared on the slide glass and also used as a control of preservation of nuclear detail whereas the other one stayed air dried first for 20 minutes and then covered with normal saline for 80 seconds before being proceeded to wet fixation.

Result: Cellularity on the slide with rehydration showed no loss in comparison with that of air-dried control. Nuclear detail was preserved with rehydration method in comparison with that of wet-fixed control. The cell size with rehydration method was larger than that of wet control. There was no blood retained on the slide with rehydration method.

Conclusion: Rehydration with normal saline for wet fixation can overcome the above-mentioned disadvantages resulting from improper processing and can bring some additional beneficial effects with enlargement of the cells and hemolysis. Both bonus effects are good for cytologic screening for tumor cells and interpretation in a bloody smear. We recommend this method be part of routine work of wet fixation for Papanicolaou stain in conventional body fluid cytology.

Disclosure of Interest: None declared.

O-105

Pleural Metastasis from Carcinoma Ex Pleomorphic Adenoma Diagnosed by Effusion Cytology: A Case Report

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Objectives: Carcinoma ex pleomorphic adenoma (Ca-ex-PA) is an infrequent salivary malignancy, arising from a primary or recurrent benign pleomorphic adenoma. Invasive Ca-ex-PAs are extremely aggressive malignancies, and up to 70% of patients develop local or distant metastasis. Pleural metastases from Ca-ex-PAs could be misdiagnosed as biphasic malignant mesothelioma or carcinosarcoma. It often poses a diagnostic challenge to cytopathologists. Herein, we report a case of Ca-ex-PA in submandibular gland, presenting as bilateral pleural effusions and lung metastases in a female patient. In order to avoid the diagnostic pitfalls, the characteristic cytologic and the immunohistochemical features are introduced.

Materials and Methods: A 54-year-old female patient with a past history of Ca-ex-PA in left submandibular gland, treated with surgery and radiotherapy, presented with progressive dyspnea and dry cough. The chest X-ray and the computed tomography demonstrated multiple metastatic tumors at bilateral lungs, accompanied by pleural effusions. Percutaneous drainage for pleural effusion was performed and the fluid was admitted for cytologic evaluation. Smears were prepared using the conventional and the liquid based methods. Moreover, additional cell block sections were also obtained for immunohistochemical study.

Results: The histologic slides of the submandibular tumor have been reviewed, and revealed Ca-ex-PA with predominantly high-grade adenocarcinoma. The Pap and Liu stained smears disclosed cohesive groups or single cells possessing variable nuclear pleomorphism, and blending into the chondromyxoid substance. The SurePath Pap slide showed mostly dispersed single cells with less myxoid stroma. Immunohistochemically, the tumor cells were positive for CEA and GATA3, but negative for TTF-1 and estrogen receptor. The immunophenotype were consistent with the diagnosis of Ca-ex-PA with pleural metastasis.

Conclusion: The diagnosis of Ca-ex-PA is problematic on the cytologic specimen. The presence of single cell populations with notable atypia, which is associated with a characteristic chondromyxoid substance, suggests the possibility of Ca-ex-PA. Immunohistochemical analysis is essential to confirm a definitive diagnosis of Ca-ex-PA in effusion cytology.

Disclosure of Interest: None declared.

O-106

A 13 Year Old Presenting with Metastatic Melanoma in Pleural Effusion Cytology

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Objectives: Occult metastatic malignant effusions are uncommon in children. We discuss a case of metastatic melanoma presenting as a malignant pleural effusion in a 13-year-old girl diagnosed by pleural fluid cytology by correlation with immunocytochemistry and confirmed by histology.

Material and Methods: A 13-year-old girl presented to the emergency department with a one-week history of feeling unwell with non-specific symptoms including upper back and shoulder pain. On examination there was decreased left sided air entry. Chest x-ray showed a large left pleural effusion. A chest tube was inserted and the aspirated fluid was sent to pathology. One monolayer stained slide and cell block were available for evaluation. Immunocytochemistry favored melanoma and a subsequent biopsy provided histological correlation for definitive diagnosis.

Results: Pleural cytology showed the presence of a largely dispersed population of pleomorphic cells with nuclear pleomorphism, binucleation, irregular outlines and prominent nucleoli. On cytomorphological features a differential diagnosis of Hodgkin's versus melanoma was entertained. Immunocytochemistry on the cell block showed the neoplastic cells to be strongly positive to S100 and Melan-A, while being CD45, CD30, CD3, and CD20 negative, confirming a melanocytic/neuroectodermal cell lineage.

Histological examination confirmed a pleomorphic mitotically active epithelioid malignant neoplasm with focal prominent pigmentation and diffuse positivity to S100, Melan A and HMB45 raising the differential diagnosis of clear cell sarcoma, PEComa and melanoma. FISH analysis for rearrangement of EWSR1 gene was negative. Metastatic melanoma was thus the final definitive diagnosis.

No primary cutaneous lesion was found (presumably due to regression) despite a dedicated physical re-examination of the patient.

Conclusions: Though malignant effusion is a common presentation of known advanced cancers, pleural effusion is an infrequent presentation of metastatic malignant melanoma. To the best of our knowledge, with detailed literature review, we present the first case of pleural effusion based cytological diagnosis of metastatic melanoma in a previously healthy pediatric patient with immunocytochemical and cytohistological correlation supportive of the diagnosis.

Disclosure of Interest: None declared.

Urine and Lymph Node

O-107

The Presence of Cytomorphological Atypia in Urothelial Tissue Fragments from Urinary Tract Washing Specimens Is Significantly Associated with Urothelial Neoplasia on Subsequent Tissue Biopsy

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Objectives: We previously have shown that the presence of urothelial tissue fragments (UTF) in voided urine specimens is associated with a small increased risk of high-grade urothelial carcinoma (HGUC) on follow-up biopsy, but only in instances in which cytomorphological atypia can be found in the fragments. The significance of these atypical UTF (AUTF) is in contrast to what has been seen when benign-appearing UTF (BUTF) are present in voided urine specimens, which only have a slight association with low-grade urothelial neoplasia on follow-up biopsy. It is not uncommon to see UTF in urinary tract washing specimens, as normal urothelial lining is dislodged in the washing process. In this study, we examine whether the presence of AUTF in a washing specimen has any clinical significance.

Materials and Methods: 1173 urinary tract washing specimens were identified over a 10-year period. 337 specimens had UTF, of which 104 had BUTF, 206 had AUTF, and 27 had UTF that were described as neither benign nor atypical. The corresponding follow-up surgical biopsy results were analyzed.

Results: The mean age was 66.9 years and 268 (22.8%) were female. 935 (79.7%) were white and 153 (13.0%) were black. When compared to specimens containing BUTF, those with AUTF were significantly associated with high-risk diagnoses of 'atypical urothelial cells, cannot exclude high grade urothelial carcinoma' or a definitive diagnosis of HGUC (34.5% vs. 0.98%, $p < 0.001$). Specimens with AUTF were significantly associated with having a follow-up tissue biopsy (64.1% vs. 39.4%, $p < 0.001$). In addition, urinary tract washing specimens with AUTF were significantly associated with a neoplastic diagnosis of LGUC (n = 9), CIS (n = 8), or HGUC (n = 60) on subsequent biopsies ($p = 0.047$). The overall rate of HGUC in specimens with AUTF was 33.0% (68/206) and 13.5% (14/104) for specimens with BUTF ($p = 0.077$).

Conclusion: These data suggest that UTF found in urinary tract washing specimens should be examined for the presence of cytomorphological atypia. Given the increased pre-test probability for HGUC among patients undergoing urinary tract washings, it is not unusual to have an increased risk of HGUC on subsequent biopsy in this cohort. However, the presence of AUTF almost triples the risk of HGUC as compared to the presence of BUTF (33% vs. 13%). Thus, the identification of AUTF in washing specimens provides useful risk stratification to the urologist and the patient.

Disclosure of Interest: None declared.

O-108

Does Combined Six Cytologic Feature System Make Urine Cytology Highly Accurate?

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Objective: To establish a guideline for urinary cytology is important for the wide use of a new reporting system for urinary cytology in Japan. Limited number of cytomorphological features effective for evaluating routine urinary cytology specimens seem necessary for wide distribution of the new reporting system. Here, we would like to demonstrate an attempt to create a highly accurate diagnostic system of urinary cytology.

Materials and Methods: We statistically analyzed 374 voided urine cytology specimens treated with Papanicolaou stain for which a final diagnosis had been made at 11 medical institutions in Japan. We evaluated the cytology using a 4 tired classification (negative, atypical, suspicious, and malignant) of the new reporting system of urinary cytology of JSCC. We observed these specimens on the presence or absence of 30 cytomorphological features. Twenty of the 30 features were extracted using a test of statistical significance and the 96 specimens (66 HGUC specimens and 30 negative specimens histologically confirmed) underwent discriminant analysis using these 20 features. And these were prepared using conventional methods, and stained with Papanicolaou's staining method. Significant differences ($P < 0.05$) were seen for twenty features among the 30 features.

Results: Six valid features such as (1) eccentric nuclei, (2) nuclear protrusion, (3) increased N/C ratio, (4) irregular chromatin distribution, (5) irregular nuclear shape and (5) hyperchromasia, were extracted from the discriminant analysis. Those 6 features obtained in this study gave 64 combination patterns. A positive predictive value investigated using all 6 features was 98.6% for cancerous specimens, and a negative predictive value was 100% for noncancerous specimens. The negative likelihood ratio (LR) was 0.02 and the positive LR was infinity. For verifying a significance of the combining six cytologic feature system, we obtained excellent results for negative LR (zero) with both the conventional reporting system categories and the new reporting system categories; however, the positive LR for the new reporting system categories was infinity, indicating that the new categories were better.

Conclusions: We developed a new diagnostic system of urinary cytology using 6 cell feature combination system, which can guarantee high positive probability of HGUC. And, the results of this investigation comply with the 4 risk stratified category system in Japan.

Disclosure of Interest: None declared.

O-109

Application of the Paris System of Urine Cytology: An Institutional Experience

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Background: Urine cytology is routinely employed for screening and surveillance of urothelial carcinoma (UC). There is a wide interobserver variability mainly ascribed to lack of an established reporting template. A convention in Paris proposed such a template with objective morphologic criteria for categorizing specimens as unsatisfactory, negative for high grade urothelial carcinoma (HGUC), atypical urothelial cells (AUC), suspicious for HGUC, HGUC and low grade urothelial neoplasm (LGUN).

Objective: We undertook this study as a first step towards assessing the predictability of TPS in identifying and grading UC.

Method: A total of 209 urine voided specimens from 80 patients evaluated from November 2014 to August 2015 were retrieved in whom follow-up biopsy diagnosis of UC were available. The cytological features were reassessed along the TPS criteria bracketing each specimen into one of the six subcategory and correlated with the final biopsy diagnosis.

Results: Males vastly outnumbered females (M:F = 9:1) with a mean age of 55.5 years. On biopsy, 50 patients (62.50%) were diagnosed with HGUC and 30 (37.5%) had low grade papillary UC (LGPUC). In our original cytological evaluation, 26 cases were categorized as HGUC while 31 patients were rendered inconclusive report where the cells shed were classified as 'atypical but not diagnostic' for carcinoma. Remaining cases were reported negative for carcinoma (18) and unsatisfactory (5) respectively. When applying TPS criteria, urine specimens were declared unsatisfactory and negative in 10 and five patients respectively. Among the 50 biopsy proven HGUC cases, cytological features in 34 (68%) patients were correctly recognized as HGUC and additional four patients (76%) were categorized as shedding cells suspicious for HGUC. The combined sensitivity and specificity of these two subcategory were 76% and 83.33% respectively. When patients with AUC diagnosis were also added, 82% of 50 HGUC patients had at least one atypical cytology report. Also 14 of our initial 'atypical but not diagnostic' cases were upgraded to HGUC subcategory. TPS criteria proposed for LGUN successfully predicted LGPUC in only nine (30%) out of 30 patients. Four and one patients were misdiagnosed to have HGUC and suspicious for HGUC respectively, seven were given diagnosis of AUC while cytological features were perceived to be negative (4) and unsatisfactory (5) in the remaining.

Conclusions: TPS has helped to standardize the urine cytology reporting at our institute. Moreover the stringent objective criteria have decoded a significant proportion of ambiguous 'atypical but not diagnostic' reports into definite HGUC. However, the successful prediction of LGPUC remained frustratingly low.

Disclosure of Interest: None declared.

O-110

The Accuracy of Urine Cytology for Low-Grade Papillary Urothelial Carcinoma

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Background: Urine cytology is a non-invasive procedure, cost efficient, and plays an important role in the screening for urothelial carcinoma (UC) and follow-up after treatment. It has been widely accepted that the sensitivity of urine cytology for high-grade UC (HGUC) is high. On the other hand, the sensitivity of urine cytology for low-grade papillary UC (LGUC), which is easily observed by cystscopy (CS), is low. This is because that the differentiation of LGUC cells from reactive cells is difficult due to absence of significant atypia. Furthermore the incidence of LGUC cells in urine is low. Fibrovascular core with capillaries is one of the important criteria for LGUC of urine cytology, but its incidence is exceedingly low. Therefore further cytological findings are needed to detect LGUC cells in urine. We focus on marked increase of intermediate urothelial cells with mild cytologic atypia as predictable cytological features for LGUC. The aim of our study is to evaluate the accuracy of urine cytology for LGUC and also consider the diagnostic significance.

Materials and Methods: 4692 consecutive cases underwent urine cytological examination in Chiba Cancer Center from January 2008 to November 2012. Cases with HGUC or urothelial carcinoma in situ (CIS) were excluded from current study. Membrane filters were used for urine specimens. Cytological results according to the presence of papillary tumor (PT) and rate of histological examination in PT were examined. In addition, Cytological results of LGUC were examined. Cytological results were divided into 2 groups: negative and positive for malignant cells. Suspicious of carcinoma cases were considered to be positive.

Results: Cystoscopically, 496 cases were diagnosed as PT, of which 42 cases had positive cytology (8.5%). In contrast, nearly all of 4196 cases without PT had a negative cytology. Of 496 cases with PT, histological examination was performed in 306 cases (62%). Median tumor sizes were 6 mm and 3 mm for cases with and without histological examinations, respectively. Histological examination revealed LGUC in 291 cases and cytological sensitivity was 14%. Tumor size of cases with positive cytology was significantly greater than with negative cytology.

Conclusions: Many low-grade PT were negative for malignant cells in urine cytology, so sensitivity for LGUC was low. However, these negative cytology (false negative) give the important information that indicates no existence of HGUC or CIS. Sensitivity of cytology for LGUC depends on tumor size, however there are some cytology positive cases with small tumor. Decrease of tumor cell cohesiveness might be related with biological grade of malignancy. We speculate that positive cytology for LGUC might be risk factor of recurrence or progression.

Disclosure of Interest: None declared.

O-111

Surface Immunoglobulin Light Chains Decisive for Diagnosing B-Cell Lymphomas; How to Assure the Best Staining Results

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Objective: Surface immunoglobulin light chains (sIg LCs) are decisive for differentiation between reactive lymphocytic proliferations and B-cell lymphomas. In-house protocol developed at our hospital for flow cytometric immunophenotyping of poorly cellular cytological samples that is daily used for determination of sIg LCs. The results were satisfactory until recently. When we look at execution of the staining protocol it was revealed that the single washing, which is performed before adding the antibodies, was omitted because of unknown reason. The aim of the study was to determine whether the number of washings performed before adding the antibodies and the type of flow cytometer (FC) used influence sIg LCs results. The influence of additional washings on cell loss during sample preparation was examined too.

Material Methods: The influence of washings on sIg LCs results and cell loss during sample preparations were tested in fine needle aspiration lymph node samples. Firstly one-wash against no-wash procedure was compared in 12 paired samples. Secondly 1, 2, 3 or 4 washings were tested in 14 paired samples. In-house protocol for FCI sample preparation was used. In each test tube 200000 cells were added. Four-color antibody panel for sIg LCs was applied (kappa FITC, lambda PE, CD19 PerCp-Cy5.5 and CD10 APC; BD Biosciences). All samples were measured on BD FACSCanto II FC. Twelve samples where 1 washing step was tested were also measured on BD FACSCanto 10c FC. In 13 samples cell loss after 1, 2, 3, and 4 washings was determined using Sysmex XP-300 Automated Hematology Analyzer.

Results: The sIg LCs staining results were significantly better when at least one washing was performed before adding the antibodies ($p < 0.004$). When number of washings was increased up to 4 times the percentage of positive kappa light chains has changed. The best results were obtained with 2 ($p < 0.023$) and 3 washings ($p < 0.010$). The percentage of positive lambda light chains remained almost the same. Regarding the type of FC used there was no significant difference when one washing step was performed ($p = 0.109$). The number of washings decreased the number of cells significantly ($p < 0.001$). During one washing on average 37.4% cells were lost.

Conclusions: The best sIG LCs staining results were obtained when 2 to 3 washings were performed before adding the antibodies, however each washing results in significant cell loss. Since a lot of cytological samples are poorly cellular, two washing steps are proposed in order to assure accurate results of sIg LCs. The type of FC used in our study not influenced the sIg LCs results.

Disclosure of Interest: None declared.

O-112

A Case of Lymphoma Cell Infiltration in the Sentinel Lymph Nodes Associated with Breast Cancer: An Importance of Papanicoloau Smear Preparation of the Lymph Nodes in Parallel

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Objectives: Intraoperative diagnosis of the sentinel lymph nodes is an indispensable process in surgical resection of breast cancer. Here we presented a case of lymphoma cell infiltration in the sentinel lymph nodes presented during the breast cancer operation. The intraoperative diagnosis was initially substantiated by cytological examination of Papanicoloau (Pap) smear preparation of the lymph nodes.

Materials and Methods: For intraoperative diagnosis of the sentinel lymph nodes, Pap smear preparation was complementarily used with hematoxylin-eosin (HE) staining of the frozen section of the lymph nodes.

Results: A 56-year-old woman was admitted to our hospital for surgical resection of the invasive ductal carcinoma of the right breast, which had been found 3 months before. She had been suffering from adult T-cell leukemia/lymphoma (ATLL) 3 years before, for which chemotherapy turned out to be ineffective. The sentinel lymph nodes were presented during the operation of the breast cancer. HE image of their frozen section of showed no evidence of breast cancer metastasis. However, their normal follicular structure was comparatively vague, mostly occupied by small-to-medium-sized lymphoid cells, which was suggestive but not by itself conclusive of malignancy. In the event of these ambiguous situations, Pap smear of the lymph nodes was prepared in parallel. The smear revealed relatively homogeneous but dissociated population of small-to-medium-sized lymphoid cells with nuclei with groove or cleavage against open chromatin. Although typical 'flower cells' were not observed, this pap image strongly suggested ATLL cell infiltration of the sentinel lymph nodes. By subsequent immunohistochemical examination of the resected lymph nodes, the final diagnosis was confirmed as infiltration of ATLL cells in the sentinel lymph nodes coexisting with invasive ductal carcinoma in the right breast.

Conclusion: Two take-home messages are presented. First, this case illustrates an importance of preparation in parallel of Pap smear of the lymph nodes in intraoperative diagnosis. HE image of the frozen section of the lymph nodes hampers precise morphological examination of intracellular structure due to various artifacts. On the other hand, Pap smear of the lymph nodes is superior in examination of nuclear details of lymphoid cells, which may be particularly helpful in small-to-medium-sized lymphoma as in the present case. Second, in intraoperative diagnosis of the sentinel lymph nodes, pathologists should pay attention not only to the epithelial components but also to the background lymphoid components.

Disclosure of Interest: None declared.

ACTA CYTOLOGICA

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Female Genital Tract (Gynecology)

there is a possibility of further complications from mixed infections.

Disclosure of Interest: None declared.

P-001

Utility of High Risk Human Papillomavirus (HR-HPV) Testing and the Influence of Vaginitis on the Cytological Findings of Patients with Atypical Squamous Cells of Undetermined Significance (ASCUS)

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Objectives: Evaluation of the utility of HR-HPV testing, the influence of mixed infection on cervical intraepithelial neoplasia (CIN), and the diagnostic accuracy of cytological findings.

Materials and Methods: Of the 4,724 patients who had a cervical cytology in our hospital from January 2014 to July 2015, 377 (7.98%) samples showed ASCUS. We retrospectively evaluated 151 of the patients with ASCUS using HR-HPV testing [cobas 4800 system, Roche Diagnostics]. We investigated the relevance of HR-HPV infection to cervical intraepithelial neoplasia (CIN) grade and diagnostic accuracy. Furthermore, we evaluated the influence of mixed infection on CIN lesions in women who reported discomfort. We then compared the diagnostic accuracy of CIN from the HR-HPV testing with conventional cytological diagnosis. The study protocol was approved by the ethics committee at our hospital.

Results: Of the 151 patients with ASCUS tested for HR-HPV, 33.1% were found to be HPV positive with rates for HPV16, HPV18, and other HPV genotypes of 9.9%, 2.6% and 27.2%, respectively. The rates for CIN2 and CIN3 in patients who underwent pathological diagnosis were 14.3% and 5.4%, respectively. The rate of CIN2 or 3 (CIN ≥2) was significantly higher in HPV16 positive patients than in HPV negative patients. The absolute risk (AR) rate of HPV positive for CIN ≥2 was 22.0% overall; the highest AR rates for CIN ≥2 were seen in HPV16 positive patients. Rates for both sensitivity and negative predictive value (NPV) for CIN3 were 100.0% with rates for CIN ≥2 of 81.8% and 86.7%, respectively. Bacterial infection was seen in 15.9% of all patients and Group B streptococcus was the most frequent bacteria. Chlamydia and gonorrhoeae were seen with rates of 3.3% and 0.7%, respectively. The CIN ≥2 rate was significantly higher in patients with mixed infection of HPV16 or 18 and bacterium or STD than in patients with only HPV16 or 18. Rates for sensitivity for CIN ≥2 was higher from the HR-HPV testing than from the cytological diagnosis alone. We found ASCUS findings to be variable amongst the cases and were not distinguishable as inflammatory responses or preneoplastic conditions.

Conclusion: The HR-HPV testing following the cytological diagnosis showed higher sensitivity for CIN ≥2 than the cytological diagnosis alone. Among women with ASCUS cytology, HR-HPV testing is an effective triage method for patients with CIN ≥2. Among the CIN ≥2 patients with HPV16 or 18 positive,

P-002

Prevalence of High Risk Human Papillomavirus Infection Using Aptima HPV mRNA Assay and Cytology Result in National Cancer Institute, Thailand

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Background and Objective: Cervical cancer is the second most common cancer in Thai woman. The main cause is high risk types of human papillomavirus (HPV). The new modality for cervical cancer screening is to combine the cervical cytology with HPV testing. Aptima HPV mRNA Assay, the new technique for detection of E6/E7 mRNA from HPV is more specific for high grade cervical intraepithelial neoplasia (CIN) than HPV DNA testing because E6/E7 mRNA identifies the presence and activity of high risk HPV infection to lead to disease. The aim of this study, we evaluate the prevalence of high risk HPV mRNA in woman compare with cytology result and correlate of age in annual check up Thai woman.

Materials and Methods: We evaluated retrospective cases from 4,476 women who come for check up programmed by combination of liquid base cytology and high risk HPV testing from January 2014 to March 2015 in National Cancer Institute, Thailand. HPV mRNA was extracted from residual specimens collected during routine liquid based cytology tests. HPV mRNA testing was performed by APTIMA HPV high risk (HR) and APTIMA HPV 16, 18/45 tests on the PATHER platform. The correlation between cytology result and age were also assessed.

Results: The overall high risk HPV prevalence was 9.29% from 4,476 women including 1.14% of HPV 16, 0.44% of HPV 18/45, 7.64% of HPV non 16, 18/45 and 0.07% of HPV both 16, 18/45. The mean age of the study group was 46.5 years (range 18–75 years). The prevalence of HR HPV infection was 17.03% in women aged 21–30 years, 12.04% in women aged 31–40 years, 6.84% in women aged 41–50 years, 7.35% in women aged 51–60 years, 15% in women aged 61–70 years and 25% in more than 70 years. The HR HPV positive found 38.7% in normal cytology and 61.3% in abnormal cytology. The HR HPV negative found 94.5% in normal cytology and 5.5% in abnormal cytology. The prevalence of HR HPV infection was found 43.8% in atypical squamous cells (ASC), 63.5% in low grade squamous intraepithelial lesions (LSIL), 93.7% in high grade squamous intraepithelial lesions (HSIL) and 37.5% in atypical gland cells (AGC).

Conclusion: In this study, The prevalence of high risk HPV infection using APTIMA HPV mRNA assay in Thai women were lower than other studies. It decreased in women aged 41–60 years and increased with cytology grade. The results of this study provide baseline information on the HPV type distribution, which may be useful for clinicians to decide who should be mon-

itored or treated more aggressively and assess HPV vaccine programs.

Disclosure of Interest: None declared.

P-003

Conventional Cervical Smear Followed by Hybrid Capture II on Slide Scrapings for Single 'Screen and Treat' Strategy. A Prospective Study of 1011 Patients

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Objective: HPV testing of cervical smear scraping is a method of combining cytology and HPV testing in a single sampling.

Materials and Methods: A prospective study with two arms was undertaken. Enrolled patients in arm one had 263 consecutive patients in whom conventional cervical smears, standard samplers for Hybrid Capture-2 (HC-II) and slides scrapings for HC-II were done. Second arm had 750 patients in whom initial conventional cervical screening and standard samplers were collected. Patients with positive cytology were triaged using HC-II performed on slide scrapings and in standard samplers.

Results: 27/263 (10.26%) smears were positive for ASCUS and above with 3 LSIL, 2HSIL and 1ASC-H. HC-II results expressed in Relative Light Units (RLU) titres were compared between scrapings and regular samplers. RLU of positive smear scrapings were on average 11.39 times less than corresponding HC-II samples, attributable to the reduced number of cells. At RLU cut off of 10 for standard samples and 1 for scrapings, there was high sensitivity (70.00%) and specificity (98.8%). Conventional cytology in 750 patients came positive in 47 cases. HC-II performed using slide scrapings at RLU cut off at 10 detected all the HPV positive cases using regular samplers. All biopsy positive HSIL or carcinoma cases were positive on slide scrapings.

Conclusions: HC-II performed on conventional cervical smear scrapings with RLU cut off >1 detects high titre positive cases which includes all the cases with significant findings. Reflex testing of slide scrapings from positive cytology smears can be used to triage for HSIL/Carcinoma. Patients with cytology and scrape tests coming positive can undergo cervical ablation without biopsy in a developing country scenario.

Disclosure of Interest: None declared.

P-004

Cervical Cytology and HPV Status in Oudomxay Province, Lao PDR

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Objective: Oudomxay province is located in the north part of Laos, where the cervical cytology is unavailable in this area. In 2012, the cervical cytology & HPV testing were conducted at six district hospitals at the capital city, Vientiane, Lao PDR by Ryukyu University. This screening in 2013 was performed to obtain the results of cervical cytological screening and HPV testing in Oudomxay Province, and then to compare them between Oudomxay Province and the Vientiane, capital area, in Lao PDR.

Method: The samples were collected from 300 healthy volunteers with the average age of 37 years (range 25–55 years) who live in the prefectural capital and neighboring cities in Oudomxay province. Ninety-six percent of the subjects were married at the average age of 22 years and 93% experienced pregnancy. The cytological diagnosis followed TBS2001. We checked the high-risk HPV infection status by both PCR assay and hybrid capture assay among the cases with abnormal cytological results.

Result: The cytological examination revealed NILM in 268 cases (90%), ASC-US in 20 (7%), LSIL in 7 (2%), HSIL in 2 (0.7%), and unsatisfactory specimens in 3. No cases with ASC-H, AGC, nor SCC were included. The high-risk HPV status examined by PCR and hybrid capture assay was positive for 45% and 40% for ASC-US cases, and 100% and 86% for LSIL cases, respectively. Two HSIL cases were positive for both PCR and hybrid capture assay. Among 28 HPV-positive cases, the most common genotype was 58 (11 cases, 39%), 16 (7, 25%), 33 (4, 14%), 18, 33, 35, 52b, 58 (1 each case, 4%), and multiple genotypes (3, 11%).

Conclusion: The number of abnormal cytological results at Oudomxay province (9.8%) is almost the same as the results obtained at Vientiane (9.3%). The most common genotype was HPV 58 (39%) in Oudomxay province, whereas that at Vientiane was HPV 16 (43%). The distribution of HPV genotype seems different between these two areas.

Disclosure of Interest: None declared.

P-005

Infection and Prevalence of High Risk and Probable High Risk HPV Types in Beijing Women

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Objectives: Human papillomavirus (HPV) has been identified as the primary cause of cervical squamous intraepithelial lesion and invasive cervical cancer. To investigate the infection situation of high risk HPV in Beijing women.

Materials and Methods: We tested 33,167 specimens from screening women and 7,499 samples from gynecology outpatients using HC2 system. The test results showed that the HR-HPV positive rate for the gynecology outpatient was significantly higher than that for screening women (30.18% vs. 13.87%). At the same time, we evaluated the sensitivity and specificity of the OriGene HPV assay. We tested 594 HC2 clinical samples using OriGene assay. The results showed that the sensitivity and specificity were 96.7% and 98.1% respectively and the overall agreement of these two assays was 97.6%. As we know, genotype prevalence is important in clinical applications, such as primary cervical screening and HPV vaccine development. In the present study, we genotyped the positive samples from Beijing women with OriGene assay using the 18 HPV individual probes.

Results: The results showed that HPV 16 was the most frequently found, with a prevalence of 31.4%. HPV-58 and HPV 52 was the second and third most common genotype, which was detected in 22.1% and 21.4% of HPV infected women, respectively. The HPV 45 and 18 are uncommon types in the Beijing area. We also genotyped probable HR-HPV positive samples with the five individual probes of the probable HR-HPV types. The results showed that the most common probable HR-HPV type was HPV 53. And then HPV 66 is second most common probable HR-HPV type. The HPV 70, 73, and 81 were very uncommon in Beijing area.

Conclusion: Our results confirmed the suggested that the HPV genotype distribution in China is quite different from other regions in the world. Therefore, some of the HPV primary screening method and vaccine development used in the other regions in the world might not fit for in China. The US FDA just approved a first human papillomavirus test for primary cervical cancer screening recently. This screening method just detects HPV 16 and HPV 18 two HR types. If this method is used in China for primary screening, the sensitivity must be too low.

Disclosure of Interest: None declared.

P-006

Detection of Human Papilloma Virus Using Hybrid Capture II and Cellprep LBC Cervical Solution

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Objectives: Department of Pathology, Seoul Natuional University Bundang Hospital has run Cellprep liquid-based cytology (LBC) system since July 2013. Because not only liquid-based cervicovaginal smear but also HPV testing is important for evaluation of uterine cervical lesions, we reviewed the results of Hybrid Capture II HPV testing using Cellprep LBC cervical solution.

Materials and Methods: We reviewed all the Hybrid Capture II HPV tests which were requested in Health Promotion Center of SBUBH using Cellprep LBC cervical solution from July, 2013 to Nov, 2015. Total number of HPV testing was 6,954 cases. We reviewed the results of HPV detection tests covering 14 types of high-risk HPV (16/18/31/33/35/39/45/51/52/56/58/59/66/68). We also evaluated technical aspects in the laboratory.

Results: High-risk HPV was positive in 518 out of 6954 cases (7.4%). Annual HPV positivity consisted of 114/1529 (7.4%) in 2013, 223/2948 (7.5%) in 2014 and 181/2474 (7.3%) in 2015, respectively. Using Cellprep LBC cervical solution, there was no technical problem encountered during DNA extraction, hybridization and acquisition of the results.

Conclusion: The Hybrid Capture II HPV tests which used Cellprep LBC cervical solution showed stable and consistent results, and we evaluate that Cellprep LBC cervical solutions are feasible for HPV DNA testing.

Disclosure of Interest: None declared.

P-007

Quantification of L1 HPV16 Methylation by Pyrosequencing. Clinical Performance in Histologically Confirmed Cases

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Objectives: To evaluate the potential of HPV16 L1 gene methylation in distinguishing precancerous lesions from invasive disease.

Materials and Methods: Our study involved the 3rd Department of Obstetrics and Gynecology, the Department of Cytopathology and the 2nd Department of Pathology, all three hosted in 'Attikon' University Hospital, Medical School of Athens University, and the Department of Obstetrics and Gynecology of University Hospital of Ioannina City. A hundred and forty five women with liquid-based cytology sample, HPV DNA test positive for HPV 16 and with histology diagnosis, were enrolled for the evaluation of methylation status of L1 gene. A total of 12 L1 HPV16 specific sites were quantified with Pyrosequencing technology. The statistical analysis was performed by SAS 9.3 for Windows.

Results: Analysis of the results showed that the mean methylation increases according to the severity of the lesion, as this is expressed by the histological outcome. The result of the ROC curve analysis, in determining the power of the methylation at various positions as a discriminator of different histological groups, depicted that the mean methylation percentage is an excellent predictor for CIN3+ lesions.

Conclusion: The findings suggest that HPV16 L1 gene methylation as quantified by Pyrosequencing technology may be a promising method to distinguish women infected with HPV16 with highest risk to harbor cervical cancer.

Disclosure of Interest: None declared.

P-008

HPV16 E2 Gene Disruptions Identified with QPCR

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Objectives: The viral E2 gene has been proposed as the most common breaking point of the viral genome prior to its integration in the host genome. However, the exact breaking site or the frequency of the breaking point still remain elusive. We created a simple real-time PCR based technique for the identification of disruptions of E2 of HPV16 in cervical samples.

Materials and Methods: 36 histological confirmed cervical smears collected and stored in ThinPrep liquid based cytology were used for DNA extraction and HPV typing using a commercially available kit (CLART2 HPV, Genomica, Spain). A region spanning about 300 bases from each side of the E2 gene was amplified and used as standard for q-PCR validation. 3 overlapping primer sets were designed for the amplification of 450–550 bp products covering the entire E2 gene. Amplification was monitored on an ABI 7500 fast q-PCR machine using the intercalating dye Syto-9. A standard curve for each product was created and the ratio of each product was calculated.

Results: The average of calculated ratios of the PCR products varied significantly with a range of 0.92–1.14 for NILMs and CIN1, 1.21–4.44 for CIN2-3 and 0.67–8.87 for carcinomas. A ratio under 0.5 for a product compared to both other two products was considered as a loss of integrity of the E2 gene at that site. In total 10/27 (37%) of CIN2+ cases displayed a partial loss of one of the three PCR products. More specifically breaking of E2 gene occurred in 2/11 (18%) of CIN2, 4/10 (40%) of CIN3 and 4/6 (67%) of carcinomas. On the other hand, loss of a product was not identified in any of the 9 (0%) cases of NILM and CIN1. Loss of either of the 3 products was possible; 4 cases lost the PCR product at the 5' end of E2, 2 cases lost the PCR product at the middle of the gene and 4 cases the product targeting the 3' end of the gene.

Conclusion: Even though E2 disruption of HPV16 is not a significantly common event and the number of analyzed samples is relatively small, the increasing incidence of E2 disruption as lesion severity progressed and the absence of any disruption in NILM and CIN1, indicates that testing for E2 disruption could be a viable ancillary test. Furthermore, since E2 disruptions seem to happen across the entire gene, methods using the E2/E6 ratio could significantly underestimate the frequency of E2 disruption.

Disclosure of Interest: None declared.

P-009

Combined E6/E7 HPV and CDKN2A mRNA Detection with Flow Cytometry

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Objectives: Since detection of the CDKN2A gene's product, p16INK4A, has been shown to have higher specificity for identifying high grade intraepithelial lesions than other HPV detection techniques, we explored the possibility of combining E6/E7 HPV mRNA with CDKN2A mRNA detection.

Materials and Methods: 157 cervical smears from a larger population of a colposcopy clinic with high prevalence of HPV were selected. Samples were collected and stored in ThinPrep liquid based cytology and were used for p16/Ki67 immunocytochemistry using a commercially available kit (CINTEC+, Roche) and E6/E7 mRNA detection (Oncotect, InCellDx) according to the manufacturer's instructions. An additional probe for CDKN2A mRNA labelled with Cy5 was added during the hybridization step and samples were analyzed on a flow cytometer equipped with a blue and a red laser. p16 ICC was evaluated by an experienced cytopathologist.

Results: The study population included 65NILM (41.4%), 24 ASC-US (15.3%), 2 ASC-H (1.3%), 55 LGSIL (35%) and 11HGSIL (7%). Positivity for E6/E7, p16 mRNA and p16 ICC was 40.4%, 2.7% and 2.1% respectively for HGSIL- and 72.7%, 72.7% and 81.8% for HSIL+. The most prominent change was observed in LGSIL were positivity for E6/E7 mRNA was almost 49%. For HGSIL positivity was not altered by including the CDKN2A mRNA result. The CDKN2A mRNA results displayed high correlation with p16 ICC ($p < 0.001$ Kruskal wallis test) while their concordance reached 98% (154/157).

Conclusion: Flow cytometry offers the great advantage of combining different fluorochromes to identify several targets in a single cell. This initial effort highlights the feasibility to combine a second mRNA probe in order to increase specificity of E6/E7 mRNA detection, while more samples with histologic confirmation are required in order to support these initial findings.

Disclosure of Interest: None declared.

P-010

P53 Codon72 Polymorphism in Women with Cervical Cancer and Precancerous Lesion

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Objectives: To investigate P53 codon72 polymorphism in women with cervical cancer and precancerous lesions.

Materials and Methods: A total of 100 exfoliated cervical cell specimens were chosen in this study. These cases were divided into group 1 (normal and LSIL, 50 cases) and group 2 (cervical cancer and HSIL, 50 cases) by pathological diagnosis. DNA was extracted and p53 codon72 region was amplified with specifically-designed primers, and the PCR products were sequenced directly and compared with the standard sequence.

Results: The result of P53 codon72 sequences showed that there were more cases with Pro/Pro genotype in group of cervical cancer and HSIL than that in group of normal and LSIL ($P < 0.05$). The risk of cervical cancer and HSIL in cases with either Arg/Arg or Arg/Pro was much lower than that with Pro/Pro by the OR value.

Conclusion: The risk for cervical cancer and HSIL of Pro/Pro genotype was much higher than that of Arg/Arg and Arg/Pro genotypes among women.

Disclosure of Interest: None declared.

P-011

Modified SurePath Processing Doubles the Available Residual Volume for Ancillary Molecular or Cytology/ICC Testing

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Objectives: The number and type of molecular ancillary tests performed out of cytology sample collection media continues to increase. Current SurePath LBC processing removes 8 ml from the collection vial to produce a cytology pellet/slide, leaving 2 ml of residual cervical specimen for ancillary molecular or cytology/ICC tests. We report here the validation of a modified SurePath LBC procedure that utilizes 6 ml to produce a cytology pellet/slide, thus doubling to 4 ml the residual specimen available for ancillary testing.

Materials and Methods: Sample processing parameters were modified on the Totalys SlidePrep instrument to compensate for the 6 ml sample volume input. These parameters ensured that the same volume and concentration of the specimen was transferred to the slide as compared to samples processed with the original 8 ml volume input. Studies were carried out to confirm that the slide quality and cellular content of the 6 ml slides were non-inferior to the original 8 ml slides. Pooled cervical cytology specimens spiked with cultured SiHa cells were used to emulate rare event

cases. Pools were spiked at a low concentration, targeting an average of 5–10 SiHa cells per slide. 96 samples were tested at each volume input level. A prospectively collected cohort was assembled by collecting two cervical specimens from 384 subjects. Each pair of samples were homogenized, equally split and then processed by the 6 ml and 8 ml methods. All resulting slides were utilized to compare the quality characteristics of Cellular Distribution, Cellular Preservation and Slide Staining. All slides were masked and randomized prior to scoring by a board certified cytotechnologist. The amount of cellular material on these slides were determined by image analysis using the FocalPoint Slide Profiler.

Results: The number of rare event SiHa cells found on the 6 ml and 8 ml slides were 6.95 (95% ci 6.45, 7.44) and 6.89 (95% ci 6.44, 7.36), respectively with a p value of 0.7923 (Wilcoxon test). 384/384 of the prospectively collected sample pairs were scored as acceptable for Cellular Distribution, Cellular Preservation and Slide Staining. The FocalPoint imaging analysis was performed by extracting the internal NUM_OBJ feature and reporting it for each slide. The mean 'NUM_OBJ' for the 6 ml and 8 ml slides were 26895 (95% ci 25821, 27986) and 25446 (95% ci 24346, 26441), respectively.

Conclusion: The data clearly show that the quality and cellular content of the new 6 ml SurePath sample processing is non-inferior in all respects to the original 8 ml SurePath sample processing. The 6 ml processing ensures that 4 ml of residual material is available for downstream molecular or cytology/ICC based tests.

Disclosure of Interest: BD employee.

P-012

Potential Impact of HPV Genotyping on Management of AGC/AECC Lesions in Newfoundland and Labrador (Nl)

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Objective: In women less than 40, 90% of cervical adenocarcinomas are due to human papilloma virus (HPV) and about 40% in women 60+ [1]. Currently in NL cervical glandular lesions are not an indication for HPV genotyping of pap smears [2]. However, the current SOGC guidelines [3] indicated that patients with negative of HPV tests without other lesions could be followed with colposcopy q12 months instead of q6 months. The objective was to determine if HPV genotyping of AGC and AEC paps in patients without another lesions would be cost effective.

Methodology: All AEC or AGC paps were received during 2013 were identified. Available cytology and surgical pathology reports from 24 months prior and post were reviewed to determine if HPV genotyping or surrogate marker of HPV infection (LSIL, HSIL, SCC, AIS, or adenocarcinoma) were present.

Results: In 2013 there were 51,565 paps. 151 (83 AEC, 68 AGC) paps were identified; representing 137 (72 AEC, 65 AGC) patients, mean age 41 (15). 35 patients had had HPV genotyping (1 HPV16, 4 OHR). Of these patients 76 (52 AEC, 24 AGC) had positive HPV test or surrogate marker of HPV infection, pending completion 31/12/2015. 18 patients had a prior glandular lesion, and would have been identified in a prior year. As a result, 56 patients (18 AEC, 38 AGC) may have had a change in management had HPV genotyping been performed.

Conclusion: Had the HPV testing on the index pap for 56 patients been negative there would have been two fewer colposcopy visits over two years for each patient. A colposcopy visit is estimated to be in excess of $7001[4], HPV genotyping is approximately $50[5], resulting in net savings of nearly $73,000 over the two year follow up period.

Disclosure of Interest: None declared.

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P-013

Cervical Human Papilloma Virus Infection in Chinese Asymptomatic Period of Human Immunodeficiency Virus – Infected Women

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Objectives: Human papilloma virus (HPV) is one of the most common causes of cervical cancer in the world, especially in China. Human Immunodeficiency Virus (HIV)-infected women as a special group of people, whose immune system severely damaged, are more susceptible to HPV, as well as the greatly increasing risk of cervical cancer. We are more interested in the rate of HPV infection in the group of asymptomatic HIV carriers in the early stages of HIV/AIDS. Whether there is a significant difference with that of normal multitude.

Materials and Methods: On October 2015, an public welfare project for HIV-infected women were carried out in Beijing Ditan Hospital.106 asymptomatic HIV carriers without antiretroviral therapy before, have undergone HPV DNA and cervical TCT tests. Meanwhile, 309 control subjects were evaluated the same projects. Abnormal participants were arranged for colposcope with multi-spot biopsy and related organization pathology inspection.

Results: The average age of HIV-infected group is 38.6 years (19–63), while the control group's average age is 34 years (18–69).

HPV DNA test was evaluated in 84 HIV-infected individuals and 250 uninfected subjects. All participants were performed cervical cytological examination. Overall 32/84 (38.1%) of HIV-infected and 23/250 (9.2%) of uninfected subjects were infected with HPV, as well as 30/84 (35.7%) and 19/250 (7.6%) had high-risk subtypes respectively. The pleomorphism mixed infection rate of HPV were 16/30 (53.3%) and 13/19 (68.4%) respectively. The top five subtypes of HPV in HIV-infected group were 52 (8/32, 25.0%), 16 (6/32, 18.8%), 18 (5/32, 15.6%), 56 (5/32, 15.6%), 66 (5/32, 15.6%), while the control group were 16 (5/23, 21.7%), 18 (5/23, 21.7%), 58 (4/23, 17.4%), 33 (3/23, 13.0%), 52 (3/23, 13.0%). There were 34/106 (32.1%) individuals with abnormal cytology in the HIV-infected group, while the control subjects was 18/309 (5.8%). The detection rate of the combination of TCT with HPV DNA tests in the HIV-infected group and control group were 37/84 (44.0%) and 28/250 (11.2%) respectively.9 cases of HIV-infected group received cervical biopsy, in which 5 cases (55.6%) were diagnosed CIN1 level, 3 cases with CIN2-3 level and 1 case with cervical microinvasive carcinoma.

Conclusion: The risk of HPV infection in the group of asymptomatic HIV carriers in the early stages of HIV/AIDS is much higher than that of non-HIV infected patients ($p < 0.01$). Sexual and behavioural risk factors do not explain the entire discrepancy in HPV prevalence between them. All the HIV-infected women, especially the asymptomatic HIV carriers should be considered as a high risk group of HPV infection. The combination of TCT with HPV DNA tests for cervical cancer and cervical precancerous lesions screening can improve detection rate.

Disclosure of Interest: None declared.

P-014

External Quality Assurance of Cervical Smears in HIV-Infected Women from Low Resource Communities

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Objectives: Human papillomavirus (HPV)-based screening has been proposed as an alternative strategy in low resource settings. The efficacy of HPV testing in HIV-infected women is uncertain. ACTG 5282 is a multicentre, randomized phase 2 trial evaluating HPV testing in the diagnosis and treatment of cervical disease in HIV-infected women at several international sites. Diagnostic modalities must be optimised in order to accurately validate HPV test results. A quality assurance (QA) programme was developed, the cytologic component of which will be discussed.

Materials and Methods: Two external cytology QA schemes were utilised. In Scheme 1, pathologists and technologists were required to participate in a commercially available QA programme viz. College of American Pathologists or Royal College of Pathologists of Australasia QAP, at baseline and then annually. Scheme 2 involved sending 5 randomly selected cervical smears to an independent central reviewer who assessed smear quality and diagnosis at baseline, and then 3 to 6 monthly for the duration of the study. Concordance between site and central diagnoses was assessed. Study sites undertook remedial activities if QA assessments proved sub-optimal.

Results: Seven laboratories (5 in Africa, 2 in India) participated. At baseline, 14 of 28 personnel had acceptable Scheme 1 results and an additional 10 passed on repeat testing 6 months later, and maintained their acceptable scores annually. Four never passed. At Scheme 2 baseline, 2 laboratories passed while 6 laboratories passed 3 years later. Most unacceptable results were due to smear collection and processing issues.

Conclusion: Sustained QA activities, in low resource settings, can make a significant, positive impact on cytologic diagnostic ability. Issues with smear collection (e.g. excessive blood, degeneration) and processing (e.g. expired reagents, pale stain), as well as attention to cytomorphology, need to be emphasised.

Disclosure of Interest: None declared.

P-015

Comparison of Liquid-Based Cytology with Conventional Papanicolaou Smears in Atypical Squamous Cells, Cannot Exclude High-Grade Squamous Intraepithelial Lesion

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Objectives: To compare Liquid-based cytology (LBC) with conventional papanicolaou smears in patients with atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H patients).

Materials and Methods: Retrospective evaluation of cervical cytological data from 49,210 patients identified 310 ASC-H patients between Sep. 2009 and Sep. 2015 at St. Marianna University School of Medicine Hospital. In ASC-H patients, the results of colposcopic biopsy and clinical diagnosis were analyzed.

Results: ASC-H patients accounted for 0.62% of cytology reports (0.61% with conventional smears and 0.64% by LBC, showing no difference between the two methods). The histological diagnosis of ASC-H patients using LBC and conventional smears was squamous cell carcinoma (SCC) in 3.3% (n = 5) vs. 2.5% (n = 4), cervical intraepithelial lesion 3 (CIN3) in 12.4% (n = 19) vs.

8.92% (n = 14), CIN2 in 11.1% (n = 17) vs. 10.2% (n = 16), CIN1 in 13.1% (n = 20) vs. 12.7% (n = 20), cervicitis in 20.3% (n = 31) vs. 22.3% (n = 35), and normal cervical tissue in 3.3% (n = 5) vs. 6.4% (n = 10). Thus, investigation of ASC-H patients revealed high grade squamous intraepithelial lesions (HSIL) (> CIN2) in 26.8% of LBC samples and 21.7% of conventional smears.

There were 98 ASC-H patients who did not undergo tissue sampling. There were 5 patients (1.6%) showing progression to CIN3 (3 by conventional smear and 2 by LBC). There were also 7 pregnant ASC-H patients (2.3%) (3 by conventional smear and 4 by LBC), in whom absence of HSIL was confirmed by colposcopic biopsy after delivery.

Conclusion: The present study showed that cytological diagnosis of ASC-H patients by either LBC or conventional smear can lead to the diagnosis of > CIN2. Many ASC-H patients did not undergo colposcopic biopsy. Five ASC-H patients progressed to CIN3, and they should have received colposcopic biopsy. In pregnant women, cytological diagnosis might lead to overdiagnosis by LBC as well as by conventional smear.

Thus, cytological diagnosis methods need to be improved for pregnant women.

Disclosure of Interest: None declared.

P-016

Improvement of Workflow and Performance by Introducing Liquid-Based Cytology in Cervical Cancer Screening Pap Test: In a Cap-Accredited Pathology Lab in Taiwan

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Objectives: Cervical cancer is women's seventh cause of death in Taiwan. The aim of current study was to solve physician's nightmare for sampling uncertainty, also to improve the quality of patient's services.

Materials and Methods: For cervical cancer screening, this study was comparing two techniques, conventional cytology (CC) and SurePath® liquid-based cytology (LBC). We collected 8,342 women who were performed SurePath® Liquid-based cytology (LBC) and 13,557 women who were directly smeared on a glass slide for conventional cytology (CC), those were staining with Papanicolaou stain and screening at Changhua Christian Hospital in Taiwan. All slides were evaluated and classified by The Bethesda System.

Result: The results of the two cytological tests were compared. The LBC smear significantly decreased in unsatisfactory rate from 3.9% to 0.2%. Positive rate were increased than CC, at 1.2% and 0.8%, respectively. The histological diagnosis of cervix was used as the gold standard compared with cytological result. In 11 cases above HSIL of CC and 17 cases over HSIL of LBC, positive predictive value (PPV) are 63.6% and 70.6%, respectively. For glandular lesion, in 21 cases above AGUS of CC and 16 cases over AGUS cases of LBC, PPV are 19% and 31.3%, respectively. For above AGUS favor neoplasm in 5 cases of CC and 2 cases on LBC, PPV are 60% and 100%, respectively. LBC had shown higher sensitivity and positive predictive value than CC.

Conclusion: LBC method can improve specimen quality, increase sensitivity and accuracy at detecting cervical epithelial cell abnormalities. This method may reduce inconvenience of cytological resampling and follow-up, improving the quality of the healthcare in Taiwan.

Disclosure of Interest: None declared.

P-017

Hyperchromatic Crowded Cell Groups in BD Surepath Liquid-Based Cytology

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Objectives: In the BD SurePath liquid-based cytology collecting cervical mucosa using a Cervex-Brush, we frequently observe dark 3-dimensional tissue fragments which called hyperchromatic crowded cell groups (HCG). HCG are characteristic findings of adenocarcinoma in situ and high grade squamous intraepithelial lesion (HSIL), but they are also observed in benign or malignant cells. In this study, we examined the frequency of HCG in HISL specimens by BD SurePath system.

Materials and Methods: Cytology specimens of 250 women categorized as HSIL were studied. They were taken from women on routine cervical check-up, collected by Cervex-Brush, and prepared by BD SurePath LBC system. The patients' age ranged from 20 and 68 years (average 38 years). The cervical biopsy was performed in 206 of 250 patients (82.4%).

Results: The histology showed benign lesions in 10 patients (atrophy in 1, immature metaplasia in 9), Cervical intraepithelial neoplasia (CIN) 1 in 43, CIN 2 in 100 cases, CIN 3 in 47, squamous cell carcinoma in 7. The diagnostic accuracy in those of lesions more than CIN 2 was 74.3%, and that of more than CIN1 was 95.0%. HCG was seen in 67.5% of HSIL.

Conclusions: HCG were frequently present in the cytology specimens with HSIL by BD SurePath system. Although HCG are easy to find on cytological screening, we must carefully differentiate them from atrophic squamous epithelium or immature metaplastic cells.

Disclosure of Interest: None declared.

P-018

Diagnosis for Liquid-Based Cervical Cytology Using Random Forest

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Objectives: Diagnosis of liquid-based cervical cytology (LBC) was influenced by subjective judgments, which is made by pathologists, resulting in the presence of high false positive rate and false negative rate of diagnosis. Therefore, Random Forest was performed on diagnosis for LBC to explore the feasibility and to seek the application value.

Materials and Methods: Total number of 523 liquid-based cytology samples were collected and diagnosed based on The Bethesda System. Morphological features of the samples were evaluated by the application of image analysis software, follow by the Weka 3.6.5 integrated Random Forest algorithm, was performed to training samples and modeling.

Results: The overall accuracy performed by random forest was 99.21% between normal group and lesions group, 99.35% between conservative treatment group and required surgery group, 89.58% between ASC-US and LSIL, and 84.82% between ASC-H and HSIL, respectively.

Conclusion: For the diagnosis of liquid-based cervical cytology, the diagnostic effect performed by Random Forest was the best while distinguishing lesions cells from normal squamous cells, and required surgery group from conservative treatment group, respectively. The diagnostic effect was general while discriminating ASC-US from LSIL, and ASC-H from HSIL, respectively.

Disclosure of Interest: None declared.

P-019

Comparison of Unsatisfactory Sample between a Conventional Smear and a Liquid-Based Cytology in Cervical Cancer Screening

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Objectives: Cervical cytology for uterine cervical cancer screening has transitioned from conventional smear (CS) to liquid-based cytology (LBC) in Korea, as advantages of LBC are highlighted. Many pathologists perceive that LBCs reduce unsatisfactory tests however some believe that the evidence to substantiate the claim is weak. Actually, there is no published study based on Korean population data comparing the unsatisfactory Pap tests between a conventional smear (CS) and a liquid-based cytology (LBC). The aim of this study is to compare the proportion of unsatisfactory specimens of CSs and LBCs at multiple institutions including general hospitals and community-based laboratories.

Materials and Methods: Each participating institution provides a minimum of 500 Pap tests for analysis. Pap tests were classified by the characteristic of participating institution as either community-based laboratory or general hospital and also by processing method as either conventional, ThinPrep, SurePath, or CellPrep. Evaluation for specimen adequacy was conducted by cytotechnologists in each institution during the routine screening work. Reasons for unsatisfactory results were classified as technical reasons, scant cellularity, and complete obscuring factors. The unsatisfactory slides were reviewed and confirmed by cytopathologists finally.

Results: Total 40816 Pap tests from 8 general hospitals and 3 community-based laboratories were analyzed. Among them, 435 tests (1.07%) were unsatisfactory. The unsatisfactory rate of LBC (1.2%) was significantly lower than that of CS (3.3%). ($p = 0.018$). Regardless of the processing method, specimens from general hospitals showed lower unsatisfactory rates than those from community-based laboratories (0.57% vs. 2.89% in LBC, and 2.12% vs. 6.05% in CS). Comparing unsatisfactory samples according to LBC method, SurePath (0.26%) showed lower unsatisfactory rate than that of ThinPrep (1.5%) or CellPrep (1.72%). Reasons for unsatisfactory results were heterogeneous in CS. Meanwhile, 66.2% of unsatisfactory LBC was due to the scant cellularity.

Conclusion: Unsatisfactory rate of uterine cervical cancer screening test is varied according to the characteristic of institution and the processing method. Unsatisfactory rate is observed in significantly low level in the LBC compared to the CS.

Disclosure of Interest: None declared.

P-020

The Specimen Adequacy in the Cervical Screening in Our Hospital

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Objectives: Evaluation of specimen adequacy is considered to be one of the most important quality assurance for cervical cancer screening with cytology. This has been recognized just recently in Japan, but the reduction of unsatisfactory specimen is still an unsolved issue in many institutions for cancer screening. And in Japan, a considerable number of institutions obtain the specimens by using of swab. Then our aim of this study is to compare the specimen adequacy and managements in the cytology obtained by swab with those by other methods such as spatula in the cervical cancer.

Materials and Methods: (Group 1) Total 2382 cases, cervical cancer screening from April 1st 2013 to March 31st 2014 was done by swab. (Group 2) Total 2339 cases visited April 1st 2014-March 31st 2015 were examined by spatula or brush. Conventional cytology was used in both groups. We calculated the rate of unsatisfactory specimens in both groups and explored the possible mechanisms to cause discrepancy between two procedures. The specimens were interpreted 'satisfactory' when they had an estimated minimum of approximately 8,000 to 12,000 well-visualized squamous epithelial cells as The Bethesda System 2001 defined. And the specimens were interpreted as 'unsatisfactory' when they did'nt have enough cells to evaluate.

Results: The number of unsatisfactory specimen was 47 (1.97%) in Group1 and there was no unsatisfactory specimen (0%) in Group 2. All of the 47 cases were evaluated unsatisfactory because the significant shortage of epithelial cells, but none of them were obscured by such as blood or inflammation significantly. And nobody of them received repeated tests in our hospital within 1 year. Within 2–3 years, the 26 of 47 (55.3%) unsatisfactory cases received screening with cytology with spatula or brush in our hospital and all of them were evaluated as 'satisfactory'. But the remaining 21 cases (44.7%) have never received Pap smear in our hospital since then.

Conclusion: Our results suggested that the using swab for sampling was one of the major critical reasons to cause unsatisfactory specimen in our hospital. We are concerned about the fact that more than 40% of the women with unsatisfactory cytology by swab did not receive Pap smear for more than 2–3 years since then. So we have to not only improve the specimen adequacy but also construct the calling and re-examination system for those with unsatisfactory cytology.

Disclosure of Interest: None declared.

P-021

Quantitative Diagnostic of Liquid-Based Cytology Using Computer Image Analysis and Classification Model

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Objectives: Established a quantitative diagnosis classification model of liquid-based cytology that had higher accuracy by computer image analysis and biostatistics technology. To provided the practical and objective methods for clinical pathologic diagnosis.

Materials and Methods: According to TBS diagnostic classification criteria, we collected five types of cells that including 1019 NILM cells, 1044 ASC-US cells, 1075 LSIL cells, 1138 ASC-H cells, 1122 HSIL cells respectively and then measured morphological parameters and colorimetric parameters for each cells with application of ImageJ 1.48 image analysis software. Finally, we used biostatistics methods liked stepwise discriminate functions, binary Logistic regression analysis and ROC curves to establish the optimal classification model which could discriminate various types of cells perfectly.

Results: Stepwise discriminate analysis had 92.7% accuracy to discriminate NILM cells from other types of cells. Blue primary color coefficient (value = 0.425) was the best parameters that could discriminate the cells between ASC-US and LSIL, and the cross-validation accuracy were 88.5% and 82.0% of the classification model which were established by using this parameter as sample inclusion criteria. Cell area (value = 85.71 μm^2) was the optimal parameters that can distinguish ASC-H from HSIL and had 96.6%, 87.0% cross-validation accuracy of the classification model which were established by using this parameter as sample inclusion criteria. Overall discriminate accuracy of classification model was 91.0% and the accuracy of each types of cells (NILM, ASC-US, ASC-H, LSIL, HSIL) were 95.0%, 90.5%, 96.9%, 84.1%, 88.0% respectively after the sample inclusion criteria were confined by blue primary color coefficient = 0.425 and cell area = 85.71 μm^2 simultaneously.

Conclusion: Our diagnostic classification model could improve diagnostic accuracy among NILM, ASC-US, LSIL, ASC-H, HSIL (95%, 90.5%, 96.9%, 84.1%, 88.0% respectively) and overall diagnostic accuracy (91.0%) based on the sample inclusion criteria were restricted by blue primary color coefficient = 0.425 and cell area = 85.71 μm^2 simultaneously.

Disclosure of Interest: None declared.

P-022

Cytomorphologic Spectrum of Lobular Endocervical Glandular Hyperplasia

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Objectives: A subset of endocervical gastric-type mucinous carcinomas (GMC) is associated with lobular endocervical glandular hyperplasia (LEGH), whose Pap smears show a spectrum of cytomorphologic atypia. In this study, we investigated and determined cytological characteristics of LEGH with/without cervical adenocarcinoma.

Materials and Methods: We reviewed the preoperative cervical smears of LEGH combined with cervical adenocarcinoma (n = 4) and LEGH without adenocarcinoma (n = 10) retrieved from the pathology files of University of Yamanashi Hospital. Conventional Pap smears were prepared using endocervical brush. All patients underwent hysterectomy. Surgical specimens of all cases were histopathologically reviewed and diagnoses were made on the basis of the World Health Organization classification. One case of adenocarcinoma was classified into GMC, one case into minimal deviation adenocarcinoma (MDA), and two cases into adenocarcinoma in situ (AIS). In two cases of LEGH without adenocarcinoma, cellular and structural atypia was observed in only partial glands. Informed consent was obtained from all patients.

Results: Monolayered honeycomb sheets of glandular cells with yellowish intracytoplasmic mucin and bland nuclei, suggest-

ing LEGH component, were found in all cases. In both LEGH with adenocarcinoma and LEGH with cellular atypia, we observed three-dimensional cell clusters, multicolored intracytoplasmic mucin, and conspicuous nucleoli of tumor cells. High crowded grouping of atypical cells with scarce mucin was only present in smears of LEGH with adenocarcinoma.

Conclusion: We concluded that three-dimensional cellular clusters, conspicuous nucleoli and multicolored intracytoplasmic mucin are the warning signs for carcinogenesis in LEGH.

Disclosure of Interest: None declared.

P-023

The Impact of the New ACOG Cervical Cancer Screening Guidelines for Adolescents: A 10-Year Retrospective Analysis

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Objectives: In 2012 the American College of Obstetricians and Gynecologists issued new cervical cancer screening guidelines. In these recommendations, women younger than 21 years no longer require Pap testing. This study aims to examine the patterns of cervical dysplasia in our patients aged 20 and under, and to determine pathologies that may be missed by this new screening schedule.

Materials and Methods: Our cytology database was reviewed from 2002 to 2011. Adolescents were defined as females under the age of 21 years who underwent Pap testing during this time period. Cytologic diagnoses were rendered utilizing morphologic criteria defined in the 2001 Bethesda Reporting System. The results were statistically analyzed utilizing Fisher's exact and chi-square tests, the latter for larger sample sizes.

Results: From 2002 to 2011, 1,026,470 Pap tests were examined. A total of 65,443 (6.4%) were from patients aged 20 years and younger. The majority (72%) were 18 years or older. 4915 (7.51%) of the adolescent cases were flagged as abnormal, compared to 31,664 (3.29%) in the general population ($P < 0.001$). The rates of ASC-H in the adolescents (0.08%) were similar to those in the general population (0.11%) ($P = 0.044$). However, the rates of ASC-US (2.52% vs. 1.42%) and LSIL (4.83% vs. 1.57%) were almost twice as high compared to the general population ($P < 0.001$). The rate of HSIL at 0.08% was half that of the older patients (0.19%) ($P < 0.001$). Subsequent biopsies yielded 222 (4.3%) CIN 2+ diagnoses in adolescents compared with 4,908 (95.7%) in the general population. 187 (84%) patients were ages 18–20 years; the remaining 35 (16%) were aged 14–17 years. In adolescents, 117 (53%) and 105 (47%) were CIN 2 and CIN 3, respectively. No invasive cancers were detected; however, there were 2 cases of biopsy confirmed endocervical adenocarcinoma in situ (AIS). Although the positive predictive values for CIN 2+ for LSIL, HSIL, and ASC-H did not differ from the general population, the positive predictive value for CIN 2+ for ASC-US was significantly lower for adolescents (12% vs. 18%, $P = 0.008$).

Conclusion: The abnormal Pap rate was significantly higher in adolescents. The cessation of Pap testing would result in the missed diagnosis of 4.3% of our CIN 2+ cases, including 2 endocervical AIS cases. Our results are comparable to those of other studies reported in the literature. Given the low percentage of high-grade lesions and the rarity of cancers in our patients under 21 years, our data supports the ACOG recommendations to delay screening in adolescents. However, the long term consequences of the delay in diagnosis of endocervical AIS in adolescents are uncertain.

Disclosure of Interest: None declared.

P-024

The Significance of Patient Reminder and Recall System and Coupon Programs in Cervical Cancer Screening in Kanagawa Prefecture

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Objectives: A patient reminder and recall system will be key to increasing the number of patients screened for cervical cancer, we evaluated whether administrators in charge quickly and accurately record patient screening histories, and clarify the role of a free coupon program launched in 2009 in achieving this.

Materials and Methods: We conducted a questionnaire-based survey among administrators in charge of medical screening in 2012 in 33 cities in Kanagawa Prefecture, concerning history taking of individual patient screening. We analyzed the relationship between changes in screening rates from 2009 to 2010 in each city and the use or non-use of patient recall systems, or the use or non-use of patient reminders in comparison with public advertising, in unscreened patients divided between a free coupon program and a routine screening program. We also investigated whether administrative records were kept for patients targeted for screening and those receiving screening, and if so, what categorical or management system was used.

Results: In unscreened participants with individual reminders, the recall rate significantly differed between those who participated in free coupon programs and those who participated in routine screening (43% and 0%, respectively). No significant differences revealed the screening rate with routine screening programs due to the use or non-use of patient reminders. However, screening rates prominently increased due to patient reminder and coupon recall implementation in 7 municipalities. 4 of 13 cities that did not implement patient reminders for routine screening conducted coupon program recall in which screening rates increased and decreased in two cities, respectively. Moreover, the screening rates increased from 2009 to 2010 in all but one of the 8 cities that implemented patient reminders for routine screening without coupon recall. Along with budgetary constraints and the inability to handle patient influx, the lack of unscreened patient lists and essential personnel to conduct patient history taking was indicated as reasons for not conducting recalls by 24% of municipal administrators, of whom no more than 53% responded that they had a complete list of patients who had been screened.

Conclusion: Patient reminder and recall systems via the free coupon programs is potentially effective in increasing screening rates, especially young patients. Owing to the short period of effectiveness of coupons, recall of unscreened patients and essential record keeping maintenance in recording patient information are key to increasing cancer screening rates.

Disclosure of Interest: None declared.

P-025

The Signisicance of Cervical Scrapping Smear in Young Women in the View Point of the Sex-Transmitted-Disease

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Objective: Most Japanease young women are interested in sex-transmitted-disease (STD), but not in uterine cervical cancer. The aim of this study is to clarify the existence of human papilloma virus (HPV) combined with STD (Chlamydia, Gonorrhea).

Materisls and Methods: Twenty-three young women complained of vaginal discharge and lower abdominal pain were performed the examination of Chlamydia, Gonorrhea and HPV by hybrid gapture method. And more, the women who were HPV positive, were examined cervical scrapping smear.

Results: In 23 cases, 7 cases were positive in Chlamydia, 4 cases were positive in Gonorrhea. 12 cases were positive in HPV. In HPV positive cases, only HPV positive case was 8 cases. Combined with HPV and Ghlamydia case was 2 cases. And combined HPV, Chlamydia and Gonorrhea was 2 cases.10 HPV positive case were examined cervical scrapping cytology. The result of cytology was 5 LSIL, 5 NILM.IN 1 case of LSIL, the diagnosis of the pathology is severe dysplasia. So conization of uterine cervix was performed.

Conclusion: In young women who don't want to undergo an examination of uterine cancer, HPV infrction is the most number of cases in CHlamydia, Gonorrhea and HPV. At this view point, uterine cervical cancer screening is important in youg women. And we should advice all young women to undergo an examination of eterine cancer.

Disclosure of Interest: None declared.

P-026

Morphometric Study to Establish Criteria of Diagnosis Chlamydial Infection in the Pap-Smear Samples

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Chlamydia trachomatis is the most common cause of curable bacterial sexually transmitted infection worldwide. Though most women with Chlamydia infection are asymptomatic or have minimal symptoms, some develop salpingitis, endometritis, pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility. It is attributed to be a risk factor for the development of cervical carcinoma. Early diagnosis and treatment of infected individuals is required to prevent the spread of the disease and severe sequelae.

Purpose: By comparing changes in cells of smears in Papanicolaou/PAP/patients with chlamydial and not chlamydial endocervicitis to assess the probably of morphometric analysis as methods of Diagnosis Chlamydial Infection.

Materials and Methods: Prospective study results in PAP smears of patients with chlamydial infection confirmed by ELISA (n = 20) and not chlamydial endocervicitis (n = 20). Morphometric analysis of type cells, nuclear and cellular area included interactive image analysis (nuclear area, convex area, perimeter, maximum and minimum radius, length and breadth, as well as nucleus form factor and elongation factor) was performed using the Olympus cellSens Standard image analysis system with exporting the results to Microsoft Excel, at magnification of x1000. Statistical data analysis was done by use of the Statistica Ver. 6 statistical package.

Results and Discussion: In the studied cases, a significant difference was found in the mean values of the morphometrical parameters of type cells, including the nuclear area and the nuclear roundness, their complexity, cytoplasmic area score were significantly different ($p < 0.05$). This values could differentiate significantly between cells in infection by Chlamydia trachomatis. The three most important cytological criteria of nuclear changes (hyperchromasia, enlargement and anisonucleosis), when quantified by morphometry, may be helpful in the differential diagnosis between cell of smears patients with chlamydial and not chlamydial endocervicitis. We conclude that nuclear morphometric evaluation of may be used as an ancillary technique in the diagnosis of atypical changes occurring in infected cells. A combination of cytomorphology and the morphometric variables assessed in this study can yield useful information on the state of infected cells as diagnostic parameters of Chlamydial infection in the Pap-smear samples.

Conclusions: In this investigation, high sensitivity of the morphometric assays were demonstrated on the samples of PAP smears of patients with chlamydial infection confirmed by ELISA and not chlamydial endocervicitis.

Disclosure of Interest: None declared.

P-027

Economic Evaluation of a Decision Support System for Referrals to Colposcopy

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Objectives: Screening tests have been widely assessed in terms of clinical and cost effectiveness. Test Papanicolaou remains the cornerstone in cervical cancer screening but guidelines suggest that

each option is assessed according to RCT data. Cost-effectiveness analysis (CEA) is a technique that compares relative costs and outcomes of alternative interventions used for the same health outcome. It facilitates the comparison of different technologies. The incremental cost effectiveness ratio (ICER) represents the average incremental cost associated with 1 additional unit of the measure of effect. ICER focuses on more expensive/effective interventions. The aim of our study was to develop and assess what would allow a more accurate way of determining women that should really proceed to further investigation during an organized screening process.

Materials and Methods: The data were selected by nationwide colposcopy units 1251 women consented to participate. For the DSS, a combinatorial approach of high throughput technologies were used (Molecular biology techniques, Bioinformatics, Computational Modelling and Artificial Intelligence). Cumulative risk scenarios, produced as outputs, were accessed in terms of cost effectiveness. Costs of each test (unit cost) and transportation cost calculated through a probabilistic allocation. Positive predictive value (PPV) and Youden's Index were determined in order to represent effectiveness. The study had certain limitations including the lack of follow-up data on women so as to evaluate the long term effectiveness. Moreover, certain costs were not incorporated into the analysis due to lack of follow-up and/or difficulty in evaluation.

Results: According to the analysis, when willingness to pay is under 1.5 euro per PPV unit, test-pap is a cost effective choice for screening. According to Youden's Index test-pap is a cost effective alternative even when society's willingness to pay is approximately 16 euro/unit.

Conclusions: Molecular tests are associated with increased effectiveness but also high costs, thus broad implementation in general population is discouraged. On the other hand, high cost could be justified in certain sub-groups with significant social interest (high risk women, populations with limited access to services).

Disclosure of Interest: None declared.

P-028

Risk of Adverse Events after Major Surgery in Women with Regular Pap Smear Tests

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Objective: Receiving regular Pap smear tests (RPST) is a healthy behavior for adult women. The purpose of this study is to investigate postoperative major complications and mortality in surgical patients receiving RPST.

Methods: Using reimbursement claims from the Taiwan National Health Insurance Research Database, we identified 702063 surgical patients with RPST and 209407 non-RPST patients undergoing major non-gynecological surgeries. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for 30-day postoperative complications and mortality associated with RPST were analyzed in the multivariate logistic regressions.

Results: Compared with non-RPST patients, women with RPST had a lower risk of 30-day postoperative mortality (OR 0.53, 95% CI 0.50–0.56). After adjustment, RPST was also associated with reduced stroke (OR 0.59, 95% CI 0.57–0.60), pneumonia (OR 0.69, 95% CI 0.67–0.72), urinary tract infection (OR 0.88, 95% CI 0.86–0.90), septicemia (OR 0.91, 95% CI 0.89–0.94), acute renal failure (OR 0.79, 95% CI 0.75–0.84), and acute myocardial infarction (OR 0.68, 95% CI 0.62–0.74).

Conclusions: Postoperative complications and mortality were significantly decreased in women with RPST. Better attitude and practice of cervical cancer screening may be the possible explanation for this phenomenon.

Disclosure of Interest: None declared.

P-029

Assessment of the Triage for Abnormal Cervical Cytology in Medical Institutions Capable of Precise Examination Post Implementation of the Bethesda System

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Objectives: We assessed the present state and issues of triage for abnormal cervical cytology in medical institutions conducted precise examination post implementation of the bethesda system (TBS) and verify the utility of TBS.

Methods: We conducted a follow up survey of 34,451 people taken a pap smear with public expense at cytological center of Kofu medical association in Yamanashi prefecture (YP) in 2013.

We investigated the result of 105 people taken detailed examination in Yamanashi prefectural central hospital (YPCH).

Results: 1) Comprehensive Survey of Living Conditions say response rate to pap test (age; 20–69, YP) was 40.2% in (3rd in total 47 prefecture). Response rate to detailed examination was 46.9% in YP.

2) Pap test results (total N = 34,451; taken a pap test with public expense).

NILM n = 33,756 (98%), ASC-US n = 365 (1.1%), ASC-H n = 40 (0.16%), LSIL n = 106 (0.31%), HSIL n = 69 (0.2%), AGC n = 14 (0.04%), AIS or Ad.Ca n = 4 (0.01%), SCC n = 1 (0.003%). Unsatisfactory for evaluation n = 93 (0.27%). Total number of abnormalities n = 599 (599/34451, 1.74%) 3,105/599 (17.5%) people take detailed examination in YPCH. The results of pap test with public expense are below (total N = 105).

ASC-US n = 29 (29/365; 8%), ASC-H n = 18 (18/40; 45%), LSIL n = 20 (20/106; 19%), HSIL n = 28 (28/69; 41%), AGC n = 7 (7/14; 50%), AIS or Ad.Ca n = 2 (2/4; 50%), SCC n = 1 (1/1; 100%). Examination In our hospital is shown. ASC-US: 26/29 patients was

conducted colposcopic biopsy (CB). The result is below. negative n = 20 (including 3 cases only cytodiagnosis (CD)), CIN1 n = 1, CIN2 n = 2, CIN3 n = 6.

ASC-H: 17/18 patients was conducted CB. The result is below. negative n = 3, CIN1 n = 1, CIN2 n = 1, CIN2-3 n = 2, CIN3 n = 9 (including pathology specimen from other hospital; PSOP). LSIL: 18/20 patients was conducted CB. The result is below. negative n = 16 (including 2 cases only CD), CIN1 n = 3, CIN2-3 n = 1.

HSIL: 25/28 patients was conducted CB (including PSOP). The result is below. negative n = 4, CIN1 n = 2, CIN1-2 n = 1, CIN2 n = 3, CIN3 n = 18 AGC: 7/7 patients was conducted CB (including PSOP). The result is below. negative n = 3, CIN3 n = 1, AIS+CIN3 n = 1, Ad.Ca+CIN3 n = 1, Ad.Ca n = 1 A case of AIS or Ad.CA and SCC patients conducted CB and diagnosed with each Ad.Ca and SCC.

Conclusion: ASC-US and LSIL tend to be managed by a primary medical institution. In case, it is persistent or getting worse, conducted precise examination in a higher order medical institution. Case of ASC-US was made triage by HPV test. TBS is useful, the accuracy of estimated diagnosis needs enhancing due to tendency to be underdiagnosis compared with histological diagnosis.

Disclosure of Interest: None declared.

P-030

Abnormal Cervical Cytology of Japanese Young Women

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Objectives: The ASCCP consensus guideline updated in 2012 recommends less intensive management for women aged 21~24 years with abnormal cervical cytology because cervical cancer risk remains low through age 25 years. The purpose of this study is to present the pathological diagnosis of young women with abnormal cervical cytology and confirm the recommendation of ASCCP guideline for the management.

Material and Methods: This is a retrospective study of women aged 21–24 years with abnormal cervical cytology between January 2004 and September 2015 at a single university hospital.

Result: We examined 80 patients of the age 21–24 years with a confirmed pathological diagnosis. Their pathological diagnoses were 19 CIN2 and 25 CIN3+ (25/80, 31.3%). 21 of 25 patients with CIN3+ were treated by invasive interventions those were one vaporization, 19 conization and one trachelectomy.

Conclusion: Over 31% women with abnormal cervical cytology aged 21–24 years had CIN3+. It is important to discuss that the management of the ASCCP guideline for young women with abnormal cervical cytology is just enough.

Disclosure of Interest: None declared.

P-031

Clinicopatholigic Findings of Patients with Atypical Squamous Cells of Undetermined Significance (ASC-US) by Pap Smear Test

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Objectives: The purpose of this study was to define the clinicopatholigic findings of patients with atypical squamous cells of undetermined significance (ASC-US) by Papanicolaou smear test.

Materials and Methods: Records of women with an index Papanicolaou smear showing ASC-US during the five years from April 2010 to March 2015 were retrospectively analyzed. In our department, patients with ASC-US underwent an immediate colposcopic examination and punch biopsy of the uterine cervix. Two hundred twelve women initially diagnosed with ASC-US were reviewed.

Results: During the 5 years, 17,485 Pap tests were performed, 15.3% of the cases, 2680 tests, suffered from cervical abnormalities in cytology; among them, 469 tests indicated ASC-US. ASC-US was detected in 1.7% of Pap tests (56 of 3,372) in 2010, but was detected in 4.2% of Pap tests (138 of 3,262) in 2014. Two hundred ninety-eight tests with ASC-US were obtained from follow-up patients with dysplasia, and 36 tests were obtained from patients with post-conization state. Among the 212 patients reviewed here, 57 women (27%) were 50 years or older. Abnormal colposcopic findings were noted in 74% of cases, and high risk human papillomavirus infection (HPV) was detected in 66%. Cervical intraepithelial neoplasia (CIN) was detected in 74% of patients (133 of 180) with ASC-US; among them, 21 cases were diagnosed with CIN 2 (moderate dysplasia of the uterine cervix). There was no CIN 2 with negative high risk HPV-DNA except for one patient. A final Pap test was negative in 74% of patients (128 of 174).

Conclusion: ASC-US is a cervical cytologic finding category suggestive but not definitive of squamous intraepithelial lesions (SIL). ASC-US remains an incompletely described entity and accounts for 5% of reported Pap tests. The management of women with ASC-US remains controversial. CIN 2 was detected in about 10%, but about three-fourths of the patients with ASC-US had negative Pap test at the final visit. The dilemma surrounding the management of patients with ASC-US diagnosis still exists, and women with ASC-US should be followed closely, especially women with high risk HPV-DNA.

Disclosure of Interest: None declared.

P-032

The Diagnostic Relevance of Neutrophils in Reactive Cellular Changes Associated with Cervical Inflammation

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The reactive cellular changes associated with cervical inflammation on cytology were researched. This may be accompanied by abundant neutrophils with reactive morphology. The characteristic of cervical inflammation associated neutrophil are poorly defined and has been considered negligible until recently because of their short life span and fully differentiated phenotype. This study aims to determine of morphology neutrophils in Papanicolaou test as a diagnostic indicator of reactive cellular changes associated with cervical inflammation. A retrospective review of Papanicolaou test slides was performed with assessment of the state neutrophils. Cases that had been diagnosed as reactive cellular changes associated with cervical inflammation without (n = 50) and with fungal organisms morphologically consistent with Candida spp and flora suggestive of bacterial vaginosis (n = 50) were studied. Slides were reviewed and scored based on death of neutrophils: necrosis, apoptosis and extracellular trap cell death (ETosis). This type of death is formed of activated neutrophils by release extracellular chromatin to form DNA traps. Data were analyzed by χ analysis. Among 100 cases the proportion of those diagnosed with reactive cellular changes with associated inflammation without microorganism increased across two categories death neutrophil – apoptosis and ETosis ($P < 0.0001$). So, a diagnosis reactive cellular change associated with inflammation on Papanicolaou test is reliably associated with type death neutrophils. As the Papanicolaou test diagnosis of morphology neutrophils and with type of its death does not correlate with clinical symptoms, but as diagnostic criterion would more reliably communicate cytology findings to clinicians.

Disclosure of Interest: None declared.

P-033

Correlation of Cytohistological and Visual Inspection Findings in Cervical Neoplasia

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Introduction: Cervical cancer is one of the commonest malignancies and a leading cause of morbidity and mortality among women. The value of Papanicolaou (PAP) smear in screening for cervical cancer has long been established and widespread PAP smear coverage can potentially reduce the burden of cervical neoplasia in Nepal significantly. Visual inspection by acetic acid (VIA) is an alternative screening method especially suited for low resource settings.

Objective: To evaluate the diagnostic value of PAP smear and VIA as methods of cervical cancer screening.

Materials and Methods: Study design: This prospective descriptive study was carried out over a period of 1 year (12.02.2012 to 12.02.2013) with a sample size of 49 cases at the Department of Pathology, Institute of Medicine, Tribhuvan University Teaching Hospital, Kathmandu, Nepal.

Statistical Analysis: The statistical test used was Pearson Chi square test and results were computed using Statistical Package for Social Sciences (SPSS) version 16.

A p-value of <0.05 was considered as statistically significant.

Collected data were statistically analyzed to determine Sensitivity, Specificity, Positive predictive value, Negative predictive value and Diagnostic accuracy of PAP smear, VIA and combined PAP and VIA.

Results: A total of 160 patients had underwent both PAP smear and cervical biopsy. Among them 49 patients had a histological diagnosis of cervical intraepithelial neoplasia or invasive carcinoma. The histopathological diagnosis of these cases were compared with cytological diagnosis. 31 of the 49 cases had VIA status available. The sensitivity of PAP smear was 61.2%, specificity 97.2%, Positive Predictive value (PPV) 90.9%, Negative Predictive value (NPV)) 85.0% and diagnostic accuracy was 86.2% in detection of cervical neoplasia. VIA had a sensitivity of 74.1%, specificity of 48.0%, PPV of 63.8%, NPV of 60.0% and diagnostic accuracy of 62.5%. On combining the two procedures sensitivity went up by 25.8%, NPV by 11.4% and diagnostic accuracy by 1.6%.

Conclusion: The present study has shown that PAP smear has a higher specificity, PPV, NPV and diagnostic accuracy but lower sensitivity than VIA. VIA by itself is not an effective screening method. A combination of PAP smear and VIA should be used in order to ensure adequate screening of cervical neoplasia.

Disclosure of Interest: None declared.

P-034

Utility of Immunohistochemistry in Cervicovaginal Cytology for Distinguishing between Endocervical Carcinoma and Endometrial Carcinoma

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Objectives: Both endocervical (endC) and endometrial (endM) carcinomas share a lot of morphological similarities, thereby making the accurate diagnosis difficult even in histological samples where immunohistochemistry (IHC) may be required for a definitive diagnosis. Herein, we report our experience using cell block (CB) preparations from residual liquid-based cervicovaginal material in adjunct with IHC to distinguish endC from endM carcinoma.

Materials and Methods: We retrospectively studied the immunohistochemical expression of Estrogen Receptor (ER), Progesterone Receptor (PgR), p16INK4a, p63, Vimentin, and Carcinoembryonic Antigen (CEA) on paraffin-embedded cell blocks from residual liquid-based cervicovaginal material of patients with his-

tologically proven endM or endC carcinomas. Among a total of 12 cases, 3 had no residual abnormal cell in the CB. The 9 remaining cases were 4 endC and 5 were endM carcinomas.

Results: EndC carcinoma showed ER expression in 0/4 (0%) cases, PgR expression in 0/4 (0%) cases, Vimentin expression in 1/4 (25%) cases (partial expression), CEA expression in 4/4 (100%) cases and p16INK4a expression in 4/4 (100%) cases. By contrast, EndM carcinoma showed ER expression in 5/5 (100%) cases, PR expression in 4/5 (80%) cases, Vimentin expression in 4/5 (80%), CEA expression in 1/5 (20%) cases and p16INK4a expression in 2/5 (40%). In all 9 cases, no p63 expression was observed. There was an absolute concordance between cytological diagnosis made after immunohistochemical staining and the final diagnosis made by histology.

Conclusion: Immunohistochemical staining for ER, PgR, CEA, vimentin and p16INK4a performed on cell block preparations from residual liquid-based cervicovaginal material is a useful adjunctive test to enhance the accuracy of the diagnosis for distinguishing between endocervical carcinoma and endometrial carcinoma. The results of this technic are as reliable as those performed on surgical biopsy material and may help to avoid the costs and the complications of a tissue biopsy.

Disclosure of Interest: None declared.

P-035

Management of Pregnant and Postpartum Women with Cervical Intraepithelial Neoplasia

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Objective: The pregnant women with cervical intraepithelial neoplasia (CIN) should be examined to role out invasive cervical carcinoma. We evaluated our diagnosis and management of pregnant and postpartum women with CIN.

Methods: After obtaining institutional review board approval, 101 pregnant patients with cytological abnormality underwent colposcopy and biopsy in the first trimester to diagnose CIN. Pregnant women diagnosed with CIN were reexamined at 2 months after delivery.

Results: One hundred one pregnant women were diagnosed with CIN in the first trimester. Fifteen (14.9%) patients were diagnosed with CIN1, 37 (36.6%) patients were diagnosed with CIN2, and 49 (48.5%) were diagnosed with CIN3. Upon postpartum reexamination, 74 (73.3%) patients exhibited no change, and 27 (26.7%) patients showed regression; no patients showed progression. Forty-one patients underwent conization, and 34 patients were diagnosed with CIN3, 5 patients with CIN1/2, and 2 patients with invasive carcinoma existed in the cervical canal. Several patients with no change at 2 months after delivery revealed regression in the reexamination at 6 months postpartum.

Conclusion: Our data suggest that pregnant women with CIN could be followed-up during pregnancy and undergo the reexamination and treatment after delivery as recommended by the ASCCP (American Society for Colposcopy and Cervical Pathology) guidelines. HPV-positive pregnant women require follow-up after delivery because their high-risk HPV types are common.

Disclosure of Interest: I have no COI.

P-036

Follow-Up Study of Pregnant Women with Cervical Intraepithelial Neoplasia (CIN)

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Objectives: Human papillomavirus (HPV) infection is known a risk factor for cervical cancer. It may be presented a hazard of progression to pregnant women with CIN. The objective of the study was to evaluate the rate of progression in abnormal cervical cytology and HPV positive pregnant women.

Materials and Methods: From January 2012 to December 2015, 4607 patients had delivery in our hospital. Ninety-three patients showed abnormal cytology. We underwent cytology, colposcopy and biopsy in first, second and third trimester of pregnancy and postpartum. We evaluated the risk factors and the course of after delivery of diagnosed abnormal cervical cytology in early pregnancy.

Results: Seventy-eight patients underwent cytology after delivery. The median age was 32 years (22 to 42). Thirty-six people diagnosed abnormal cytology before pregnancy. HPV test was undergone in 59 patients. Forty-five patients were HPV positive. The number of ASC-US, LSIL, HSIL, ASC-H, and AGC in first trimester was 31, 32, 24, 6, and 1. During pregnancy, abnormal cytology was disappearance in 27 patients. Two patients were diagnosed with invasive cancer during pregnancy. One patient underwent conization in pregnancy and hysterectomy after delivery. Abnormal cervical cytology disappeared in 45 patients, persisted in 25 patients. Ten patients underwent conization after delivery. Five patients underwent radical hysterectomy after delivery, 3 patients were after 1 month, and 2 patients were after 1 year. There is infection of HPV 16 or 18 in 13 out of 16 patients who received surgery.

Conclusion: We found no patient progressing to invasive cancer during pregnancy. Case of abnormal cervical cytology in early pregnancy would be expected usual management by the full term birth. However, there are progressing cases with high-risk HPV, especially genotype 16 or 18. This object suggests that HPV testing with cervical cytology in prior or early pregnancy may be a strategy to manage patients with CIN. The testing results will be useful for follow-up after delivery.

Disclosure of Interest: None declared.

P-037

Express Method for Detection of Cervical Pathology

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Objective: High-risk human papillomavirus (HR-HPV) is the main etiological factor in development of cervical cancer. The aim of our study was to compare the results of HPV testing using GeneXpert HPV and Cobas on cytological material with cervical pathology and to study HPV genotype contribution in development of high-grade cervical intraepithelial neoplasia. Both real-time PCR assays detect 14 types of high risk HPV. Xpert HPV is cartridge-based random-access platform which provides individual HPV16, HPV18/HPV45 genotyping with a simultaneous result for 11 other high-risk HPV genotypes (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68). Xpert HPV testing can be completed in 1 h.

Materials and Methods: Liquid-based cytology (B&D, TriPath) of 80 women were PAP tested and evaluated using Bethesda classification. Residual material was used for 14 HR HPV DNA detection by the cartridge-based PCR Xpert HPV and Cobas. P16(INK4A)/Ki67 double immunocyto-histochemical staining (CINtec PLUS Cytology Kit) were performed. Biopsies were taken in cases of L-SIL and H-SIL.

Results: HPV DNA testing results were obtained in 78 samples (22 H-SIL, 21L-SIL, 17 ASC-US, 18NILM) and were matched in 77 of 78 cases (98.7%), two samples were invalid. Cobas test was negative in one case L-SIL (histology CIN I), while Xpert was positive (11 HR HPV). Xpert HPV test revealed in 22 H-SIL: 13 women (59.1%) had HPV16, 6 women (27.3%) had multiple HPV infections (11 HR HPV). 2 women (9.1%) had combination of HPV16 and multiple HPV. In one H-SIL (4.5%) test showed combination of HPV16 and HPV18/45.

Conclusion: Xpert HPV is a rapid and reliable method for detecting HPV DNA high risk types in cytological samples and can be used not only in diagnosis but also in screening for precancer and cancer of the cervix. We noticed that HPV16 is etiologically dominant in development of high-grade cervical intraepithelial neoplasia.

Disclosure of Interest: None declared.

P-038

B-Cell Lymphoma of the Uterine Cervix in a Breast Cancer Patient Diagnosed with Liquid Based Cytology and Cell Block Technique

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Objectives: Definitive cytomorphologic diagnosis of non-Hodgkin lymphomas of the uterine cervix is possible but can be challenging in routine cervicovaginal cytology specimens. Herein, we describe how a less invasive cervical smear in a woman led to the diagnosis of B-cell lymphoma. The residual material from liquid based cytology (LBC) and cell block preparation can potentially facilitate the additional testing to improve the diagnostic accuracy.

Material and Methods: A 67-year-old female patient with a past history of breast cancer, treated with partial mastectomy, chemotherapy and radiotherapy three years prior, presented with fever and tarry stool passage. The laboratory data showed severe thrombocytopenia, as well as elevated LDH and CRP levels. The subsequent abdominal and pelvic CT demonstrated multiple metastatic tumors at liver, stomach, pancreas, right kidney, retroperitoneal and bilateral pelvic lymph nodes. A large mass at uterine cervix was also found. Owing to the high risk of massive bleeding, less invasive tests such as voided urine and cervical smear with LBC were thus performed. Moreover, additional cell block sections were obtained from the residual material of LBC specimen.

Result: The histologic slides of the breast tumor have been reviewed, and revealed invasive carcinoma with mixed ductal and lobular features. The urine cytology disclosed scant atypical cells with nuclear hyperchromasia. The ThinPrep Pap slide showed dispersed single cells forming small clusters. The atypical cells exhibited high N/C ratio and scant cytoplasm. Subsequent immunostains were also performed. The tumor cells were positive for LCA (CD45RB) and CD20, but negative for CD3, cytokeratin (AE1/AE3), pax-8, estrogen receptor and CD56. A very high Ki-67 index was also noticed (>90%). The final diagnosis of cervical lesion was a high-grade B-cell lymphoma.

Conclusion: Lymphoma of the cervix is a relatively rare tumor, and accounts for less than 1% of cervical malignancies. Primary cervical lymphoma presents a diagnostic dilemma. Small single cells in cervical cytology always pose a challenge, particularly under the suspicion of metastatic disease. The differential diagnoses include both benign and neoplastic processes, such as follicular cervicitis, endometrial cells, small cell carcinoma, and metastatic lobular carcinoma. An alternative less invasive approach such as LBC may allow further diagnostic tests in limited cytological material. We also demonstrate that cell blocks prepared from residual LBC materials provide additional information for these cytologically difficult cases.

Disclosure of Interest: None declared.

P-039

Cytologic Characteristics of Primary Cervicovaginal Malignant Melanoma: Report of Two Cases

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Objective: Primary cervicovaginal melanoma constitutes a rare disease of female gential tract and is seldom diagnosed on cervical cytology screening tests. The infrequently occurring malignancy is highly aggressive and usually associated with a high risk of recurrence. In recent ten years, we experienced two cases of pri-

mary cervicovaginal melanoma, and demonstrated their cytological features in the Pap smears.

Material and Methods: The first case was a 70-year-old woman complaining of post-menopausal vaginal bleeding. Pap smears followed by partial vaginectomy was performed. The second case was a 61-year old woman with an itching sensation over the vulva and a palpable nodule noted at the anterior vaginal wall. Pap tests and biopsy showed melanoma. Radical vulvectomy was performed.

Results: The Pap smears revealed high cellularity in a background of tumor diathesis. The malignant cells were either isolated or clustered. Tumor cells were spindle to epithelioid, with oval to pleomorphic nuclei and often had very prominent single nucleoli. The nuclear membrane was irregular with coarse chromatin. Bi- or multi-nucleation and intranuclear pseudoinclusions were also identified. Cytoplasm was scant with high N/C ratio. Occasionally fine, brown granularity of melanin pigments was found.

Conclusion: Although cervicovaginal mucosa is a rare site for melanoma, cytopathologists should always keep this entity in mind while interpreting the Pap tests. The differential diagnosis includes many poorly differentiated malignant neoplasms, either primary or metastatic. Early diagnosis is essential in order to warrant a better prognosis.

Disclosure of Interest: None declared.

P-040

A Case of Glassy Cell Carcinoma of Uterine Cervix

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Objectives: Grassy cell carcinoma (GCC) is a poorly differentiated adenosquamous carcinoma, comprising about 1–2% of all cervical cancers. We report the cytologic features of a GCC case.

Materials and Methods: The 40-year-old patient was diagnosed as stage IB uterine cervical cancer. She underwent radical hysterectomy as primary treatment. Touch smear cytology of the surgically removed uterine cervix was obtained.

Result: A large number of atypical cells, including large and round nuclei with coarse chromatin, made clusters arranged in sheet on the specimen. Prominent nucleoli were centrally located in the nuclei, and cytoplasm showed granular or 'ground-glass' appearance. Eosinophils, plasma cells and Charcot-Leyden crystal also appeared on the specimen. From these findings, we diagnosed this tumor as glassy-cell carcinoma of the uterine cervix.

Conclusion: GCC is a variant of adenosquamous carcinoma. If a smear has the features of adenosquamous carcinoma, we have to watch carefully for the distinctive characteristics of GCC such as 'ground-glass' appearance of the cytoplasm and co-existence of eosinophils, plasma cells, and Charcot-Leyden crystal.

Disclosure of Interest: None declared.

P-041

A Case of Cervical Intraepithelial Neoplasia (CIN), the Diagnosis Was Suspected by Urine Cytology

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Objectives: The diagnosis of cervical intraepithelial neoplasia (CIN) is usually triggered by abnormal findings observed on cervical smear. Here, we present a case of CIN, the diagnosis was suspected after urine cytology.

Materials, Methods and Results: A 46-year-old woman, G1P1, with proteinuria, came to our hospital for an internal medicine consultation. After spontaneous urine collection, the urine cytology showed abnormal cells which made us suspect a uterine cervical disease. As a result, gynecology was consulted and CIN 3 was diagnosed by colposcopy and biopsy.

Urine Cytology Findings: Many superficial squamous cells were observed. Small number of intermediate or parabasal squamous cells with high nucleus/cytoplasm (N/C) ratio were also observed.

Cervical Cytology Findings: With a background of neutrophils, small parabasal cells with an increased N/C ratio were observed. Nuclear enlargement, anisokaryosis, hyperchromasia and irregular distributed chromatin made us suspected HSIL:CIN3.

Colposcopy Findings: Mosaic pattern and acetowhite epithelium were observed.

Cervical Biopsy Findings: Abnormal cell proliferation was observed in all layers of the squamous epithelium. However, stromal invasion was not noticed.

With all the previous findings, CIN3 was diagnosed.

Conclusion: We presented a case of CIN, the diagnosis was suspected by urine cytology. It is possible that the urine was contaminated with abnormal cells from the vaginal secretions.

On urine cytology screening, it is necessary to consider the origin of abnormal cells not only from the urinary tract but from other organs.

Disclosure of Interest: None declared.

P-042

Metastatic Papillary Renal Cell Carcinoma in Liquid-Based Cervical Cytology: A Case Report

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Objects: The detection of metastatic renal cell carcinoma in cervical smear is a rare and late event. Furthermore, cytologic findings of metastatic papillary renal cell carcinoma in liquid-based cervical cytology specimen have not been described before.

Materials and Methods: A 46-year-old female presented with vaginal discharge for a month. The patient underwent right radical nephrectomy due to renal cell carcinoma, papillary type, nine years ago. She has been receiving chemotherapy since 15 months ago because multiple tiny lung nodules and lymphadenopathy, which were suspicious for metastasis, were detected on abdominal CT during follow-up. The liquid-based cervical cytology showed singly scattered or rare small clusters of large round to ovoid atypical tumor cells admixed with benign cervical squamous cells. The clusters were two-dimensional. The tumor cells had abundant cytoplasms, occasionally bi- or multilobated or bizarre nuclei, and/or macronucleoli, some cells resembling Reed-Sternberg cells. The tumor cells were positive for both vimentin and PAX-8. On punch biopsy specimen of the uterine cervix, poorly cohesive malignant tumor cells with the same cytomorphology as atypical cells in the cervical cytology were found within the lymphovascular space, expressing vimentin and PAX-8. The tumor cells showed focal immunoreactivity to P504S and no immunoreactivity to PAX2, renal cell carcinoma antigen, HMB45, and CD10. Histological diagnosis was 'consistent with metastatic carcinoma, clinically from the kidney'.

Conclusion: Metastatic renal cell carcinoma may be detected in a cervical cytology smear. The tumor cells are characterized by singly scattered and rarely clustered markedly enlarged cells with occasional bi- or multinucleated cells and macronucleoli. PAX-8 immunocytochemical staining can be most helpful in differential diagnosis of metastatic renal cell carcinoma from other intra-or extrauterine adenocarcinoma in liquid-based cervical cytology specimen.

Disclosure of Interest: None declared.

P-043

Cytological Diagnosis of Primary Malignant Melanoma of the Vagina : A Case Report

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Introduction: Primary malignant melanoma of the vagina is an extremely rare disease, especially in Asian women. It accounts for less than 3% of women with primary malignant tumors of the vagina. Vagina melanoma is a highly aggressive tumor, with an early local recurrence, frequent metastases to the lymph nodes and also life threatening hemorrhage. The prognosis of this tumor is very poor and overall survival rates are very low. Independent of the treatment given, overall survival of five years is 5–25%. Therefore, timely detection by Pap smears is very critical to initiate immediate treatment.

Case Report: A 82-year-old female patient presented with an abundant yellowish discharge and abnormal vaginal bleeding for 20 days. The Pap smear shows many malignant tumor cells arranged singly or in syncytial clusters. The tumor cells have pleomorphic nuclei, contain binucleate and multinucleate cells. The tumor cells show finely or coarsely irregular chromatin, irregular nuclear membranes, and prominent single or multiple nucleoli. A few single or clusters of spindle cells are also present in a necrotic background. Melanin pigment is present in tumor cells and macrophages. Histopathological examination further confirmed the case of malignant melanoma of the vagina. Immunohistochemical stains revealed positive for HMB-45 and Melan-A marker, supporting the diagnosis.

Conclusions: Primary malignant melanoma of the vagina is an extremely rare with worse prognosis, but early diagnosis of this aggressive tumor could improve the prognosis of this disease.

Disclosure of Interest: None declared.

P-044

Immunohistochemical Evaluation of High Grade Neuroendocrine Carcinoma of Uterine Cervix

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Objectives: High grade neuroendocrine carcinoma of the uterine cervix (HGNCUC) is a very rare disease representing only 1% to 5% of all uterine cervical cancers. HGNCUC progresses rapidly to hematogenous metastases, and has a poor prognosis. HGNCUC should be diagnosed promptly to determine the treatment strategy. Insulinoma associated protein 1 (INSM1) is one of zinc-finger transcription factor. Recently, it has been reported that protein INSM1 is expressed in multiple neuroendocrine tumors. However, no previous reports have evaluated expression of INSM1 in HGNCUC. This zinc-finger transcription factor should be proved as useful and reliable for diagnosis of HGNCUC when compared with chromogranin A (ChrA), synaptophysin (Syn) and NCAM.

Materials and Methods: Patients diagnosed with HGNCUC between 1985 and 2013 at Shizuoka Cancer Center, Shizuoka and Keio University Hospital, Tokyo were enrolled in the study. At least two pathologists reevaluated histological diagnosis in patients with small cell carcinoma (SmCC) or large cell neuroendocrine carcinoma (LCNEC). We evaluated INSM1 expression in

HGNCUCs by immunohistochemical analysis and compared the results with other three neuroendocrine (NE) markers (ChrA, Syn and NCAM). Thirty seven formalin fixed and paraffin embedded HGNCUCs (29 SmCC and 8 LCNEC) were used for evaluation. The cases used for immunohistochemical control were those evaluated as non-neuroendocrine carcinoma (non-NEC) (11 squamous cell carcinoma (SqCC), 6 adenocarcinoma and 3 adenosquamous carcinoma of uterine cervix), those diagnosed as lung cancer with surgical resection (5 small cell lung cancer and 5 SqCC of lungs) and lung cancer cell lines. INSM1, ChrA, Syn and NCAM were detected as using EnVisin (DAKO) immunohistochemistry (IHC).

Results: Immunohistochemically, INSM1 in HGNCUC was expressed in 35/37 (95%), in 27/29 (93%) cases of SmCCs and 8/8 (100%) cases of LCNEC. In contrast, INSM1 was not expressed in any non-NEC and SqCC of lungs, respectively. ChrA, Syn and NCAM were expressed in HGNCUCs in 32/37 (86%), 32/37 (86%) and 25/37 (68%), respectively. INSM1 expression was detected specifically in cell nuclei without any cytoplasmic stain. The sensitivity and specificity of INSM1 in HGNCUC were higher than the other NE markers.

Conclusion: Compared to traditional NE markers, INSM1 has a high prevalence and the distinction of positivity from negativity was clear cut. This methodological procedure of IHC of INSM1 will be applicable not only in the diagnosis of HGNCUC but also in that of all neuroendocrine cells and tumors.

Disclosure of Interest: None declared.

P-045

Large Cell Neuroendocrine Carcinoma of the Uterine Cervix Misdiagnosed as Adenocarcinoma in Cervical Liquid Based Cytology

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Introduction: Large cell neuroendocrine carcinoma (LCNEC) is a rare tumor in uterine cervix. Cervical LCNEC has an aggressive behavior and unfavorable outcome even in its early stage. Few cytopathologic features of cervical LCNEC have been reported. Case 1: A 45-year-old woman presented with intermediate vaginal pruritus and foul odor for 20 months, and cervical malignancy with stage IB1 was detected by pelvis MRI. The liquid-based cytologic specimen demonstrated ball-like tumor cell clusters in a necrotic backgrounds. The tumor cells had large and hyperchromatic nuclei, prominent nucleoli and moderate amount of cytoplasm, and showed nuclear overlapping with focal nuclear molding. The findings of cervical smears suggested an adenocarcinoma. The histologic and immunohistochemical examination of cervical biopsy sample revealed cervical LCNEC of the uterine cervix. Case 2: A 53-year-old woman who had been diagnosed as atypical glandular cells in routine cervical screening test at local clinic. She had gone through the cervical cytologic examination again at our hospital. The liquid-based cytologic specimen showed similar morphologic features to case 1, such as ball-like tumor cell clusters with tumor diathesis, large cells with hyperchromatic nuclei and prominent nucleoli with focal nuclear molding. The cytologic diagnosis was adenocarcinoma. The cervical conventional slide from the local clinic had been read again. It demonstrated a definite rosettoid pattern in a necrotic background and tumor cells with large nuclei, fine chromatin pattern, crushing artifact and nuclear molding. This cytologic features are compatible with that of neuroendocrine carcinoma. The histologic and immunohistochemical examination of uterine cervical biopsy also revealed cervical LCNEC.

Conclusions: Cervical LCNEC can be confused with adenocarcinoma in cervical smear, particularly liquid-based samples. Cytopathologists should pay attention to the cytopathological features of cervical LCNEC, such as rosettoid pattern and nuclear molding, to differentiate from the poorly differentiated adenocarcinoma.

Disclosure of Interest: None declared.

P-046

Primitive Neuroectodermal Tumor of the Uterus – A Case Report

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Objectives: Primitive neuroectodermal tumors (PNETs) were first described in 1973 as a group of small round cell tumors that appeared to have developed from neuroectodermal cells. PNETs are usually seen along the central axis, particularly in the soft tissue and bone structures of the chest and abdomen of young adults and adolescents. We experienced uterine PNET in older female. We reported the cytological feature in smears and histopathological and immunohisochemical results and discussed.

Case: The patient is a 96 year old female with a history of endometrioid adenocarcinoma ten years prior to admission. She was treated with hormone therapy. She complained abdominal discomfort, and admitted to her primary care physician. She was diagnosed ileus and referred to our hospital. The symptoms of ileus was reduced by the physical treatment, but atypical genital bleeding was found. She was referred to Gynecologic department. Cervical cytology was performed. The result was Positive. Small round cells were found. And then, cervical biopsy was done. Pathological diagnosis was PNET. Unfortunately, she died 10 days after.

Cytological Findings: Cytologic examination of the smears showed a hypercellular specimen that consisted of singly dispersed round cells with a high nuclear-to-cytoplasmic ratio. Neoplastic cells were round-to-oval, slightly pleomorphic nuclei with slightly irregular nuclear membranes, fine, pale chromatin and 1 or some prominent nucleoli, and lighter light-green staining cytoplasm. Some cells have moderate amounts of finely vacuolated cytoplasm.

Pathological Findings: Biopsy specimen showed highly packed atypical small round cells with blood vessels and geographical necrosis. Histological characteristics of the neoplastic cells were as same as cytological smears.

Immunohistochemical Stains: Tumor cells were CD99(+), NSE(+), Vimentin(+), Synaptophysin(+), p53(+), AE1/AE3(–), ER(–), p63(–), Melan-A(–), HMB-45(–), AFP(–), LCA(–), Desmin(–), Chromogranin A(–), CD56(–), CD10(–), and c-kit(–).

Conclusion: The cytological diagnosis of uterine PNET remains difficulties. The diagnosis of PNET in uterus is often difficult in spite of the well characterized morphological features and this is probably attributed to a low index of suspicion. It is helpful to understand PNET in uterus when diagnosing with careful evaluation of cytological features. Immunohistochemical stains are helpful.

Disclosure of Interest: None declared.

P-047

Atypical Polypoid Adenomyoma of the Uterus; Clinicopathological Findings of 6 Cases

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Atypical polypoid adenomyoma (APAM) is uncommon, benign polypoid lesion of the uterus, and usually occurs in reproductive and premenopause women. Histologically, APAM is characterized by the admixture of atypical endometrial glands and hypercellular smooth muscles in a stroma.

Objective and Method: Six patients with APAM during eight years in our hospital were studied retrospectively to invest clinical history, pathological study, and background.

Results: The age of the patients ranged from 31 to 78 years (mean age, 46 years). All patients complained about atypical bleeding, and one patient was developed severe anemic symptom by hypermenorrhea. And three cases were characteristically polypoid and two cases in which one case transformed malignant were cystic by MRI. They except one were not diagnosed until primary surgery. In one case, initial smear cytology of the endometrium showed overlapping, highly atypical glandular cells with squamous metaplastic cells combined with abundant, spindled smooth muscle cells in the stroma, which suggested APAM. Three patients were held by curettage, two patients were held by hysteroscopic transcervical resection in which recurrence case is two, while one person underwent total hysterectomy and bilateral salpingo-oophorectomy and was revealed APAM with leiomyosarcoma.

Conclusion: Most cases of APAM were benign, in some cases APAM may be difficult to distinguish from endometrioid adenocarcinoma or malignant tumor, and may recurrent tumor again. The treatment may be depend on the patient's age, fertility, and clinical issue.

Disclosure of Interest: None declared.

P-048

Endometrial Cytological Features of Well Differentiated Endometrial Carcinoma and Dysfunctional Uterine Bleeding in Liquid-Based Preparation

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Objectives: The aim of this study was to evaluate the cytological characteristics between well differentiated endometrial carcinoma (WDEC) and dysfunctional uterine bleeding (DUB). The pap smears were made by SurePath™ liquid-based preparation (LBP).

Methods: Cytological features were analyzed on a total of 75 smears (42 EC and 33 DUB) that were histologically confirmed. The cytological abnormalities about background, cellularity, cellular architecture, and cell atypia (alterations in nucleus and cytoplasm, and mitosis) were estimated.

Results: The 42 smears of WDEC were interpreted as 32 (76%) carcinoma or atypical (endometrial) cells, 1 (2%) normal endometrial cell and 9 (21%) NILM without endometrial cells on review. All 33 smears of DUB had endometrial cells. WDEC showed significant differences from DUB in the architecture of overlapping pattern (44% WDEC and 0% DUB), uneven internuclear distance within cell group (31% WDEC and 3% DUB) and the absence of condensed stromal cells (0% WDEC and 73% DUB), whereas there were no significant differences in background (clean, histiocyte, bloody and necrotic), cellularity and architectures (loss of polarity, compact or loose patterns, and individual cells). WDEC revealed severe cell atypia compared to DUB in nuclear enlargement that was more than 2 times of the nucleus in the intermediate squamous cell (24% WDEC and 3% DUB), anisokaryosis that was a twofold and more in size variation (50% WDEC and 27% DUB), less irregular nuclear membrane (52% WDEC and 82%), round nuclear membrane (88% WDEC and 55% DUB), hyperchromatic pattern (30% WDEC and 9% DUB), large nucleolus (21% WDEC and 0% DUB), presence of mitosis (15% WDEC and 0% DUB) and intracytoplasmic neutrophils (73% WDEC and 33% DUB), whereas there were no differences in normochromic pattern (15% WDEC and 63% DUB), hypochromic pattern (82% WDEC and 36% DUB), and absent or small nucleoli (75.8% WDEC and 100% DUB).

Conclusions: Combined cytological evaluation, especially nuclear features can be useful in the cytological differentiation between EC and DUB. These findings may be related with LBP which can improve the detection of endometrial cells with the preservation of nucleus.

Disclosure of Interest: None declared.

P-049

ALDH and CD44 Expression: Potential Cancer Stem Cell Maker for Differentiating Endometrial Lesions and Is Associated with Poor Outcomes in Patients with Endometrial Carcinoma

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Objectives: Cancer stem cells play essential roles in tumor metastasis and contribute to remarkably negative clinical outcomes. Recently, aldehyde dehydrogenase (ALDH) and CD44 positivity was identified as a marker of breast cancer stem cells. We evaluated expression of ALDH and CD44 protein in normal endometrium, endometrial hyperplasia and endometrial carcinoma, and investigated the prognostic value of ALDH and CD44 expression in patients with endometrial carcinoma (EC).

Materials and Methods: We conducted a hospital-based retrospective review of ALDH and CD44 distribution immunohistochemically in 245 samples of endometrium from biopsy or hysterectomy. ALDH and CD44 immunoreactivity was classified into low- and high-score or negative- and positive-score groups based upon the extent and intensity of staining.

Results: A 'high' ALDH score was observed in a high percentage of samples of EC (44.25%), compared to normal endometrium (4.76%), endometrial hyperplasia without atypia (16.67%) and in endometrial atypical hyperplasia (11.90%). A 'positive' CD44 score was observed in a high percentage of samples of EC (35.40%), compared to normal endometrium (4.76%), endometrial hyperplasia without atypia (2.08%) and in endometrial atypical hyperplasia (4.76%). There was more ALDH and CD44 expression in EC, compared to premalignant endometrial lesions. Importantly, a higher combine ALDH and CD44 score in cases of EC was correlated with poor overall survival, with a hazard ratio of 4.61 for death (95% confidence interval, 1.54–13.78).

Conclusion: Our results indicate that the prevalence of $ALDH^{high}/CD44^{+}$ tumor cells in EC is significantly associated with worse prognostic factors and favors a poor prognosis. ALDH and CD44 expression is also a potential histopathology biomarker for the differential diagnosis of malignant and premalignant endometrial lesions.

Disclosure of Interest: None declared.

P-050

The Comparison of Biological Properties of Endometrial Cancer Stem Cells Isolated Using a Violet Laser and a Near-UV Laser

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Objectives: Cancer stem cells (CSCs) possess the ability for self-renewal, differentiation and tumorigenesis, and play a role in cancer recurrence and metastasis. In the flowcytometry, CSCs are usually sorted into side population (SP) cells using ultraviolet (UV) laser (350 nm) excitation. However, it is difficult to avoid cell damage using a UV laser. Therefore, we attempted to isolate CSCs using a violet laser (407 nm) excitation to avoid cellular DNA damage.

Materials and Methods: We sorted SP cells and main population (MP) cells from a human endometrial cancer cell line (poorly differentiated endmetrioid adenocarcinoma; G3, HEC-50B cells) using the FACSAria system equipped with a violet laser or a near-UV laser (375 nm), and analyzed the biological properties of these cells.

Results: Violet-SP cells exhibited self-renewal and high-tumorigenicity. It was found that KRAS oncogene expression was significantly higher in SP cells than in MP cells.

Conclusion: Our results suggest that CSCs exist in the SP fraction sorted using the FACSAria system equipped with a violet laser, which presents a useful tool to isolate small populations of viable putative CSCs and can be used to identify and characterize CSCs.

Disclosure of Interest: None declared.

P-051

Image Analysis and Multi Layer Perceptron Neural Networks for the Discrimination of Benign from Malignant Endometrial Lesions

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Objectives: Objective of this study is to investigate the potential of a Multi Layer Perceptron (MPL) Artificial Neural Network (ANN), for the discrimination of benign from malignant endometrial nuclei and lesions.

Materials and Methods: In this study 416 liquid based endometrial cytology samples, taken with the EndoGyn® Sampler, were involved. The cases were histological confirmed. From each cytological slide nuclear morphometric features were extracted from about 90 nuclei using a custom image analysis system (in total 38,326 measured nuclei). Subsequently nuclei from 50% of the cases were used to train an MPL ANN in order to classify each nucleus as benign or malignant. The remaining data from the 50% of the cases were used to evaluate the MLP ANN performance and stability on unknown data. On the top of the nuclei classification results from the MLP ANN, a second classifier was used; this was based on the percentages of benign and malignant nuclei of each case. This second component classified individual cases as benign or malignant. The golden standard was the histological examination result.

Results: According to the analysis, the MLP ANN had Sensitivity: 69.38%, Specificity: 88.84%, PPV: 79.64%, NPV: 82.18% and OA: 81.33% (cumulative results on the training and test set) and no statistical significant difference was observed between the training and test set. The second subsystem had the following performance indexes on the classification of individual patients: Sensitivity: 99.42%, Specificity: 93.44%, PPV: 91.44%, NPV: 99.56% and OA: 95.91% (cumulative results on the training and test sets). The complete system misclassified one case of hyperplasia with atypia as benign and 16 benign cases as malignant (6 cases with histologicaly confirmed polyp, 4 cases of hyperplasia without atypia, one case of disordered endometrium, 3 cases of proliferative and 2 cases of atrophy).

Conclusions: Computerized systems based on ANNs combined with image morphometry have the potential to discriminate benign from malignant endometrial nuclei and lesions.

Disclosure of Interest: None declared.

P-052

Usefulness of Intra-Operative Touch Imprinting Cytology of Sentinel Lymph Nodes in Endometrial Cancer for Improving Intra-Operative Diagnostic Accuracy

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Objectives: Detecting method for sentinel lymph nodes (SNs) in endometrial cancer has not been established yet. We evaluated the diagnostic accuracy of touch imprinting cytology (TIC) of SNs in endometrial cancer patients.

Materials and Methods: We performed SN mapping for 105 patients with endometrial cancer without apparent extra-uterine spread who needed to receive pelvic lymph node dissection. We principally used both radioisotope method with hysteroscopy (submucosal injection) and dye-guided method (ICG, subserosal injection), and then performed hysterectomy, bilateral salpingo-oophorectomy and back-up lymph node dissection. SNs were submitted for frozen section (FS) and anti-cytokeratin immunostaining was performed with paraffin section. In 31 patients, TIC was performed for maximum cut surface of SNs before submitting frozen section.

Results: In 106 patients, SN was detected in 98% (103/105) patients. Based on the final diagnosis with immunostaining, sensitivity, specificity and negative predictive value (NPV) were 100%, 100%, 100%, respectively. However, based on intraoperative diagnosis with FS, sensitivity, specificity and NPV were 71%, 100%, 92%, respectively. Among 22 patients with metastatic SNs, FS revealed false-negative in 83% cases of isolated tumor cells, 50% in micrometastasis and 11% in macrometastasis. Among 31 patients examined also by TIC method, 7 patients (23%) showed metastasis in SNs. In FS, sensitivity and NPV were 71% and 92%, respectively, while in combined method of FS and TIC, 86% and 96%, respectively, in per-patient analysis.

Conclusion: SN navigation surgery has a problem in intra-operative diagnostic accuracy, which might be improved by combined method of FS and TIC.

Disclosure of Interest: None declared.

P-053

The Evaluation of Diagnostic Methods for the Intrauterine Recurrence Following High Dose Hormonal Therapy for Endometrial Cancer

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Objectives: High dose medroxyprogesterone acetate (MPA) therapy is a fertility preserving therapies for younger patients with endometrial cancer (EC) and atypical endometrial hyperplasia (AEH). One of the most serious problems is the high intrauterine recurrence rate following MPA therapy. The aim of our study is to evaluate diagnostic methods for intrauterine recurrence following MPA therapy.

Patients and Methods: Our study recruited 175 patients (64 AEH and 111 EC) that were treated with MPA therapy due to preserve fertility at our institution from 1998 to 2012. The eligiblity criteria of MPA therapy were as follows: 1) strong desire of fertility preserving, 2) pathological diagnosis was AEH or endometrioid adenocarcinoma G1, 3) without myometrial invasion diagnosed by MRI, 4) neither lymph node metastasis nor distant metastasis diagnosed with CT (PET-CT), 5) obtained written consent. Oral administration of MPA was continued until pathological tumor disappearance. After tumor disappearance patients were allowed to attempt pregnancy and endometrial cytology using Endocyte ® and curette biopsy was performed every 3–6 months due to checking intrauterine recurrence. We evaluated the accuracy of endometrial cytology and endometrial biopsy from 92 patients who had intrauterine recurrence following MPA therapy.

Results: Twenty seven patients (42%) with AEH and 65 (59%) with EC relapsed after MPA therapy. The recurrence rate was significantly higher in patients with EC than that with AEH. The diagnostic methods which provided the initial detections of intrauterine recurrence of atypical endometrial hyperplasia were endometrial cytology, endometrial biopsy, and both methods in 10%, 60%, and 30% of cases with AEH, respectively. They were endometrial cytology, endometrial biopsy, and both methods in 6%, 51%, and 36% of cases with EC, respectively. False-negative rate of endometrial cytology for a diagnosis of recurrence disease following MPA therapy was significantly higher than that for a diagnosis of initial disease; it was 60% in AEH and 52% in EC for a diagnosis of recurrence following MPA therapy, while was 21% and 3% for a diagnosis of initial disease, respectively. False-negative rate of endometrial biopsy for a diagnosis of recurrence was significantly lower than that of endometrial cytology; it was 10% in AEH and 5% in EC.

Conclusion: An endometrial biopsy should be performed as the diagnostic method for detecting intrauterine recurrence following MPA therapy because the false-negative rate of endometrial cytology was high.

Disclosure of Interest: None declared.

P-054

A Study of Peritoneal Cytology in Stage I Endometrial Cancer

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Objectives: The prognostic impact of positive peritoneal cytology (PC) in endometrial cancer (EMC) is still controversial, although it was dropped from the criteria in the revised 2008 FIGO staging system. Recently, histological risk factors are used to select the candidates for adjuvant therapy, and observation is often considered for low-risk patients regardless of the status of PC. We aimed to evaluate whether PC should be a part of independent risk factor for recurrence in stage I EMC patients.

Materials and Methods: We conducted a retrospective study including 526 patients diagnosed with stage I EMC who underwent hysterectomy, bilateral salpingo-oophorectomy, and lymphadenectomy between 2001 and 2012 at Jikei university hospitals. We divided them into three groups: a low-risk group (endometrioid carcinoma G1/G2 and depth of invasion less than 50%, no cervical involvement, no lymph-vascular space infiltration [LVSI]), a moderate-risk group (endometrioid G1/G2 and depth of invasion greater than or equal to 50%, endometrioid G3 and depth of invasion less than 50%, non-endometrioid without myometrial invasion, no cervical involvement, positive LVSI), and a high-risk group (others). We investigated the relationship between PC status and recurrence rate as well as the factors including histological type, stage and LVSI. Fisher's exact test and chi-square test were used for the statistical analysis.

Result: PC was positive in 43 of 526 patients (8.2%). Univariate analysis revealed the significant correlation between positive PC and the recurrence: the recurrence rate was 14.0% (6/43) in those with positive PC while it was 4.35% (21/483) in those without it. There was no significant difference in PC status and the recurrence rate among three groups. PC was positive in 6.5%, 9.4% and 8.0% in low-, moderate-, and high-risk group, respectively. There was no correlation between PC status and the pathological factors. Among the patients in low- and moderate-risk groups without adjuvant therapy, there were 6 patients with positive PC, in which 1 patient recurred.

Conclusion: Positive PC correlates with recurrence in patients with stage I EMC. Although, it is no longer a part of the current FIGO staging criteria, PC status should be taken into account when selecting the candidate for adjuvant therapy for the EMC patients. In addition, it is important to note that a subset of stage I patients of low- or moderate-risk groups may recur when PC is positive.

Disclosure of Interest: None declared.

P-055

Clear Cell Carcinoma Endometrium in Cervical Cytology

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Objectives: Clear cell carcinoma (CCC) of endometrium is rare. Its prevalence is about 1–6% of endometrial cancers. It occurs commonly in postmenopausal women without exposure to DES or HRT. The tumour is not related to obesity or hypertension. The aim of this report is to share a case of an endometrial clear cell carcinoma diagnosed on liquid based cervical cytology. Histopathology correlation is provided.

Materials and Methods: The clinical details, cytology and histopathology slides were retrieved from laboratory information system. The slides were reviewed with the pathologist and diagnosis reconfirmed.

Results and Conclusion: Cytologic features of endometrial clear cell carcinoma will be shown and differential diagnosis will be discussed.

Disclosure of Interest: None declared.

P-056

The Methods to Prepare the Slides from Endometrial Samples and the Diagnostic Criteria

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Introduction: At our institution, we started using Masubuchi-type aspirator or Endocyte to prepare the sample from the endometrium since 1993. For interpretation, instead of using conventional criteria based on detection of cellular atypia, we emphasize the observation of the structural changes. Herein, we summarize the diagnostic criteria we use.

Sample Preparation: The sample obtained was washed in a test tube containing 5 ml of physiological saline. After centrifugation at 1,500 rpm for 5 minutes, buffy coat was aspirated using a pipet. The cellular components were placed on a glass slide, and using another slide on top. They were crushed and smeared by the regular pull-apart method. After fixation in 95% alcohol, Papanicolaou stain was used, and the slides were ready for interpretation.

Cytological Appearance: We observe the following structural changes.

1. To look for papillary cell clusters.
2. Is there any branching of the papillary cell clusters?
3. The presence or absence of arborescent cell clusters.
4. Further observation of the primary or secondary branching of arborescent cell cluster.

An arborescent cell cluster is defined as a finding in which epithelial cells are connected at right angles to a type A stroma which serves as an axis inside a multilayered epithelial cell cluster in which glandular cavities are observed back to back or as a finding in which a type A stroma runs through a multilayered epithelial cell cluster.

For complex hyperplasia, usually primary branching is observed. For complex atypical hyperplasia, secondary or further branching is observed and the number of layers of type A stromata are less than 6. If the number is greater than 6, endometrioid adenocarcinoma is more likely.

Conclusion: Using the saline washed sample to prepare slides for endometrial cytology, three dimensional structures can be easily observed under the microscope. Observation of these structural changes are paramount important for differentiation of the various lesions in the endometrium.

Disclosure of Interest: None declared.

P-057

Results of Endometrial Cytology at Genki Plaza Medical Center for Health Care

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Introduction: In the past, criteria used for endometrial cytology were mainly based on the cellular atypia. Since we initiated a new procedure, and emphasized the importance of observing the structural changes, we increased the detection rate of abnormal endometrial cytology. In this communication, we analyzed the results of the study on endometrial cytology.

Material and Methods: We used Masubuchi-type aspirator or Endocyte to prepare the sample from the endometrium. The sample obtained was washed in a test tube containing 5 ml of physiological saline. After centrifugation at 1,500 rpm for 5 minutes, buffy coat was aspirated using a pipet. The cellular components were placed on a glass slide, and using another slide on top. They were crushed and smeared by the regular pull-apart method. After fixation in 95% alcohol, Papanicolaou stain was used, and the slides were ready for interpretation.

From April, 1993 to March, 2015, 739,402 patients underwent Pap smear screening for cervical cancer. Of 37,410 patients, endometrial samples were simultaneously obtained for endometrial cytology.

The incidence for endometrial cytology and the positive rate were calculated. Correlation study was performed between the cytology results and the histological diagnosis.

Results: At our institution, the examining rate of endometrial cytology was 5.1% with 144 cases interpreted as ' positive'. Of 144 cases, histological examination was available for comparison in 99 cases. (68.8%). The results histological diagnoses were as follows; normal 1, complex hyperplasia 6, endometrioid carcinoma grade 1, 67, grade 2, 11, grade 3, 2, clear cell carcinoma 1, serous adeno-

carcinoma 1, sarcoma 3, ovarian serous adenocarcinoma 2. The incidence for uterine cancer was 0.22%. The correlation between endometrial cytology and histology with complex hyperplasia or beyond was 93.3%.

Conclusion: The correlation between endometrial cytology and histology with complex hyperplasia or beyond was good. We emphasize the importance of observing the structural changes, instead of conventional criteria based on detection of cellular atypia. With new practice of managing the endometrial cytology, we have obtained a remarkable improvement in detection rate and accuracy of the endometrial cytology.

Disclosure of Interest: None declared.

P-058

A Case of Co-Existence of Small Cell Neuroendocrine Carcinoma and Endometrioid Carcinoma Arising from the Uterine Body Diagnosed by Cervical Smear as a Supplemental Method

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Background: A case of co-existence of small cell neuroendocrine carcinoma and endometrioid carcinoma arising from the uterine body is rare entity with a frequency of less than 1% of endometrial carcinomas. We report a case of uterine body carcinoma composed of small cell neuroendocrine carcinoma and endometrioid carcinoma diagnosed by cervical smear as a supplemental method.

Case: A 57-year-old-Japanese woman, gravida 1, para 1, consulted our hospital with a complaint of continuous lower abdominal pain during 1 month. Engrossed and nonmobile uterus was palpable by the physical pelvic examination. Colposcopic examination revealed no gross abnormality of the cervix or vagina. NSE was 134 ng/mL with high level on admission. MRI showed an irregular thickening of endometrial mucosa and an approximately 5 cm-sized ovary replaced by the tumor. In addition, metastases of para aortic, pelvic lymph node and right adnexa were suspected, and it suggested an advanced uterine body cancer. Positron Emission Tomography (PET) showed no malignant tumor other than the uterine body, the right ovary and lymph node described above. Cervical cytology showed small aggregates of small round atypical cells with nuclear molding and scant cytoplasm, with coarse and salt-pepper-like chromatin and inconspicuous nucleoli, suggesting small cell neuroendocrine carcinoma. Endometrial biopsy indicated well differentiated adenocarcinoma. Therefore we performed a debulking surgery and sampled lymph nodes. The resected specimen showed an irregular thickened mucosa of the uterine cavity and a 6x5 cm-sized right ovary with a white-grayish solid cut surface. Its histological features showed that endometrial carcinoma on the endometrium invaded the muscular layer with the tiny nests of necrosis, converting the small cell neuroendocrine carcinoma on the invaded lesions and the right ovary, and the transitional area between them was seen. In addition, the neoplastic cells also invaded the right round ligament and left ovary. Immunohistochemically, neuroendocrine markers (CD56, synaptophysin and chromogranin A) expressed small cell neuroendocrine carcinoma, whereas, those markers were negative in the endometrioid carcinoma component. The spreading conditions of carcinoma disclose the uterine body cancer stage3c (FIGO 2008). The patient received 8 cycles of chemotherapy in total with irradiation. The patient was disease free 14 months after surgery.

Conclusion: A diagnosis of the present case was confirmed by cervical cytology and endometrial biopsy. The cervical smear was a supplementary method to know the exact and detail histological diagnosis of the uterine endometrial carcinoma.

Disclosure of Interest: None declared.

P-059

Concurrent Endometrial Cancer and Ovarian Cancer Presenting as Pseudo-Meigs' Syndrome: A Case Report

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Objectives: Pseudo-Meigs' syndrome is often characterized by pleural effusion and/or ascites caused by a pelvic tumor, other than an ovarian fibroma. The combination of pseudo-Meigs' syndrome, and concurrent endometrial cancer and ovarian cancer is a rare condition, and it makes difficult to choose adequate chemotherapy regimen. We reported a case of concurrent endometrial cancer and ovarian cancer, presenting as the clinical features of pseudo-Meigs' syndrome: complex pelvic mass and bilateral pleural effusion.

Materials and Methods: A 71-year old woman was admitted to the emergency department with complaints of dyspnea and abdominal fullness. Computed tomography (CT) revealed bilateral pleural effusion, a 20-cm sized tumor in her pelvis, and an abnormally thickened endometrium. Magnetic resonance imaging (MRI) revealed a huge ovarian tumor. A preoperative endometrial biopsy revealed uterine endometrial cancer. Cytological examination of the pleural fluid was negative. Total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed under suspicious of the endometrial cancer with ovarian metastases presenting as pseudo-Meigs' syndrome.

Results: Cytological analysis of the uterus revealed isolated atypical endometrial cells with increased nuclear-to-cytoplasmic (N/C) ratio and hyperchromatic nuclei. Histopathological analysis of the uterus revealed grade 3 endometrioid adenocarcinoma. On the contrary, cytological analysis of the ovary was found a rosette-like formation by large cells. Immunohistochemical staining of the ovary revealed the following: CD56 (+, partial), CK7 (+), ER (+), chromogranin (-), synaptophysin (-). Thus ovarian tumor was diagnosed to be large cell neuroendocrine carcinoma with poorly differentiated adenocarcinoma, suggesting that the ovarian tumor is not metastases from the endometrial cancer, but rather the original cancer. The patient had complete resolution of the pleural effusion postoperatively. Paclitaxel and carboplatin (TC) chemo-

therapy was chosen, so that it could be effective both on endometrial cancer and ovarian cancer.

Conclusion: To our knowledge, there has been no published literature documenting the concurrent endometrial cancer and ovarian cancer presenting as pseudo-Meigs' syndrome. Thorough pathological examination of each tissues is needed to choose proper adjuvant chemotherapy. Although concurrent cancer presenting as pseudo-Meig's syndorome is a very rare condition, clinicians should always bear in mind that double cancers presenting as pseudo-Meigs' syndrome can exist and ensure the pathology of each tissues.

Disclosure of Interest: None declared.

P-060

Fine Needle Aspiration Cytology of Metastatic Partial Hydatidiform Mole

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Objectives: To describe the cytomorphologic and immunocytochemical findings of one case of partial hydatidiform mole metastatic to the lung and the difficulties to demonstrate such an origin.

Materials and Methods: A 46-year-old female was attended because of an episode of hemoptysis. X-ray revealed a 3.9 cm solid, nodular lesion in the lower lobe of the left lung. She referred an antecedent of a spontaneous abortion 18 months before managed in other center. CT-guided fine needle aspiration cytology of the pulmonary lesion was performed. Smears were stained with Diff-Quik, Papanicolaou and immunocytochemical markers. Once reviewed the curettage specimen and after the cytological diagnosis a complete gynecological examination and determination of serum markers were carried out, revealing β-hCG serum titles of 2410 mIU/ml. Residual tumoral masses were not demostrated either in the uterine cavity or in other locations.

Result: Smears showed trabecular and plaque-like hypercellular groups composed of polygonal cells with enlarged, hyperchromatic, mitotically active, pleomorphic nuclei with occasional macronucleoli and variabily densed, sometimes vacuolated cytoplasms with well-defined boundaries set in a hemorrhagic background without necrosis. Intact villi or fragments of hydropic stroma were not identified. The cells were negative for CK7, CK20, TTF1, CDX2 and GATA-3 and showed intense positivity for CK8-18, β-hCG and β-catenin (diffuse) and for p57 and p63 (patchy). Weak positivity for estrogens was detected in isolated cells. Review of the previous curettage specimen showed enlarged, hydropic villi with focal hyperplastic trophoblastic transformation that showed features superimposable to those of the cytological smears.

Conclusion: The diagnosis of metastatic partial hydatidiform mole requires the morphological and immunohistochemical demonstration of different subpopulations of trophoblastic cells in an extrauterine location. The absence of hydropic stroma or complete villi in cytologic smears does not exclude this unfrequent event, inasmuch as even in surgical specimens they can be hardly identified. Since after curettage serum β-hCG titles can return to normal levels and residual molar tissue can be absent of the uterine cavity, to know the antecedent of a previous molar gestation is essential for the correct diagnosis. Considering that choriocarcinoma shares a similar composition of different trophoblastic subpopulations, the possibility of this malignant neoplasm, developed during or after the molar pregnancy, should always be taken into consideration and accurately excluded by an exhaustive gynecological examination.

Disclosure of Interest: None declared.

P-061

Pre-Operative Diagnosis of Incisional Endometriosis by Fine Needle Aspiration Cytology

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Objective: Incisional endometriosis (IE) or endometriosis occurring in a surgical scar remains a rare and underreported entity. The incidence ranges from 0.03–1% in women following obstetric or gynecologic surgeries. As the imaging modalities are non-specific and because most lesions of IE present as a cutaneous or subcutaneous mass, fine needle aspiration cytology (FNAC) appears to be a valuable tool in providing rapid and accurate preoperative diagnosis, obviating the need for other procedures.

Materials and Methods: A total of 8 cases of cytologically diagnosed and histologically confirmed IE diagnosed between 2009 and 2014 comprised the material for this study. The patients ranged from 28 to 44 years of age. All patients had prior abdominal operations (one abdominal hysterectomy for fibroid, one laparoscopic myomectomy and six cesarean sections). They presented 10 months to 8 years later with pain and swelling in or around the abdominal scar. Two patients also complained of cyclical increase in their symptoms.

Results: The cytomorphological spectrum comprised of glandular epithelial cells and spindle or ovoid stromal cells. Hemosiderin laden macrophages were seen in four cases. Mild epithelial atypia was observed in one case. A diagnosis of endometriosis was provided in all the eight cases on FNAC and later confirmed on histopathology.

Conclusion: IE is a potential diagnostic pitfall in the approach to anterior abdominal wall masses. A safe and reliable FNAC diagnosis is possible in the light of clinical history and awareness of minor morphologic alterations in the various endometriotic components. The importance of FNAC also lies in excluding other benign and malignant masses thus permitting correct patient management.

Disclosure of Interest: None declared.

P-062

Primary Serous Adenocarcinoma of the Fimbriated End of Fallopian Tube with Peritoneal Dissemination: Report of a Case Highlighting the Cytodiagnostic Difficulties

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Case Report: The patient was a 62-year-old woman with a history of pulmonary tuberculosis and chronic hepatitis C. She visited our hospital with abdominal distension and diarrhea. Blood tests showed an elevated CA125 level (2,448 U/mL). Imaging studies revealed marked ascites and mass lesions in the mesentery, greater omentum, and peritoneum. However, there was no apparent ovarian enlargement, and upper and lower gastrointestinal endoscopy showed no evidence of malignancy. Open abdominal surgery was performed to confirm the diagnosis. At laparotomy, numerous disseminated lesions (10–20 mm) were found on the surface of the peritoneum and serosal surface of the intestine. The cancer had invaded the serosa of the uterus, mainly in the recto-uterine and vesico-uterine pouches. The ovaries were not enlarged, but disseminated lesions were seen on their surface. Intraoperative ascites cytology revealed many tumor cell clumps against the background of lymphocytes. The tumor cells exhibited papillary configurations with circularly enlarged nuclei of various sizes, increased chromatin, prominent nuecleoli, abnormal mitoses, and vacuolated cytoplasm. Psammoma bodies were also noted. Cytologically, this case was diagnosed as serous adenocarcinoma. She received postoperative chemotherapy for stage 3c peritoneal cancer (5 courses of taxotere and carboplatin), resulting in a decrease in the CA125 level to 37 U/mL. PET-CT showed no abnormal uptake. Open completion debulking surgery was performed. At laparotomy, no ascites was present, and a few disseminated lesions (several mm in size) were found on the surface of the peritoneum, small intestine, and ovaries. Partial omentectomy, supra-vaginal hysterectomy, and bilateral adnexectomy were performed. Features consistent with tubal intraepithelial carcinoma (TIC) were seen in the epithelium of the fimbriae of the right uterine tube, suggesting the site of origin of serous adenocarcinoma.

Summary: The cytological differential diagnosis of primary serous adenocarcinoma of the fallopian tube is complicated. In the present study, a patient with peritoneal cancer underwent laparotomy, and was diagnosed with serous adenocarcinoma of the fallopian tube by postoperative specimen. This case is described with reference to the literature.

Disclosure of Interest: None declared.

P-063

Relation between Hyaluronan Synthesis and Cell Morphology in Ovarian Clear Cell Carcinomas

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Objectives: Hollow spheroids ('mirror balls') are often found in the ascites of patients with ovarian clear cell carcinoma (CCC). In spite of the absence of stromal cells, hollow spheroids contain various extracellular matrix, and one of the major components is hyaluronan. It has been suggested that tumor-derived hyaluronan plays a significant role in the formation of the hollow spheroids. The purpose of this study was to clarify the relation between hyaluronan synthesis and hollow spheroids in ovarian CCC.

Materials and Methods: A hyaluronan inhibition assay was performed on HAC-2 cells *in vitro*. HAC-2 is a CCC cell line that has a high capacity for hyaluronan synthesis, as well as the formation of hollow spheroids. HAC-2 was grown in a monolayer or suspension under treatment with 4-methylumbelliferone (MU), an inhibitor of hyaluronan synthesis. Hyaluronan synthesis was evaluated by ELISA or cytochemistry, and cell morphology was observed by phase-contrast microscopy. Twenty-eight archival ascites cytology specimens (14 CCCs and 14 serous carcinomas) were also examined for hyaluronan expression cytochemically.

Results: When hyaluronan synthesis was inhibited by MU, HAC-2 failed to form hollow spheroids. Other effects of MU included the degeneration of cell membranes where hyaluronan has just been synthesized, and reduction of cell growth. In archival ascites cytology specimens, CCCs expressed hyaluronan more frequently than serous carcinomas (11 of 14 vs. 3 of 14, $p < 0.05$). Hyaluronan was predominantly detected in the cell membranes, suggesting that hyaluronan synthesis still takes place in floating tumor cells.

Conclusion: Tumor-derived hyaluronan is essential for the formation of the hollow spheroids, and is also responsible for the cell growth in ovarian CCCs. Since hyaluronan is expressed in floating CCC cells, the inhibition of hyaluronan could be a potential adjunctive therapy for CCCs with positive ascites.

Disclosure of Interest: None declared.

P-064

CD44v Expression in Ovarian Cancer Cells Derived from Ascites Is Associated with Chemosensitivity and Prognosis of Ovarian Cancer Patients

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Objectives: Malignant ascites are found in more than 30% of patients with ovarian cancer. CD44 is a cell surface glycoprotein that influences cell-cell interaction and cellular motility. This mol-

ecule undergoes alternative splicing to generate standard form (CD44s) and splice variants (CD44v). In contrast to CD44s, some CD44v isoforms play pivotal roles in aggressive proliferation and metastasis of cancer cells. Since cancer cells in ascites are implicated in peritoneal dissemination and recurrence, we analyzed association between CD44v expression in ovarian cancer cells derived from ascites and prognosis of ovarian cancer patients.

Materials and Methods: With the approval of the institutional review board, 11 fresh ascites samples from ovarian cancer patients were applied to the study. The cancer cells were isolated from ascites via erythrocyte hemolysis. Expressions of CD44 mRNA were measured by RT-PCR followed by agarose electrophoresis. Cell viability was assessed by ATP assay after 72-hours of carboplatin (CBDCA) and paclitaxel (PTX) treatment.

Results: Various levels of CD44v expression were observed in ovarian cancer cells derived from ascites: 2 cases with high expression, 3 cases with moderate expression, and 2 cases with low expression. We detected 3 types of CD44v in ovarian cancer cells derived from ascites, which consisted of variable exons v6-v10, v7-v10 and v8-v10. We found that the ovarian cancer cells derived from ascites with high expression of CD44v were lower sensitive to both CBDCA and PTX, whereas those with low expression of CD44v exhibited higher sensitivity to both drugs. Moreover, the ovarian cancer patients with high expression of CD44v in ovarian cancer cells derived from ascites showed worse prognosis, compared with the patients with low CD44v in the cells.

Conclusion: The present study indicated that expression level of CD44v could be one of the major determinants of response to chemotherapeutics. In addition, given the fact that the ovarian cancer cells derived from ascites with higher CD44v are insensitive to CBDCA and PTX, this study indicated that CD44v expression level in ovarian cancer cells derived from ascites could be utilized to diagnose prognosis of ovarian cancer patients.

Disclosure of Interest: None declared.

P-065

Identification of Allelic Losses in Sporadic Epithelial Ovarian Cancers

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Objectives: The somatogenetic alterations that lead to the development ovarian carcinomas are not well understood. This study investigated loss of heterozygosity (LOH) in sporadic ovarian cancer. Loss of tumor suppressor genes may allow for the expression of tumorogenecity or to tumor progression.

Materials and Methods: DNA samples from 54 sporadic ovarian cancers (15 early stage I/II and 39 advanced stage III/IV), 13 borderline tumors and 13 benign were analyzed using microsatellite markers. Genetic regions o chromosomes 1p32-36, 7q31-53, 11p15, 17q12-21 and 18q23 were analysed by polymerase chain reaction.

Results: Among benign tumors LOH was found in only 15.4% (at 1p), 23.1% (at 7q) and 15.4% (at 18q), allelic losses in borderline tumors were noted in 30.7% (at 1p), 38.5% (at 7q), 23% (at 11p), 30.7% (17q) and 23.1% (18q). The frequency in invasive ovarian carcinomas were found higher in 17q (79.6%) and 7q (51.8%), but lower in other lochi. Serous subtypes of invasive carcinoma more prone to deletions than non serous tumors. Comparing the results with clinical parameters, was found that allelic loss frequently in advansed stages cancers and poor-differentiated tumors. FIGO stage and the presence of LOH at 11p15 correlated significantly with survival.

Conclusions: These findings suggests that regions on chromosome 17q21, 7q31-53, 11p15 can play important role in development and progression sporadic ovarian carcinomas.

Disclosure of Interest: None declared.

P-066

p53-mut Protein as a Prognostic Factor in Patients with Epithelial Ovarian Cancer

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Objectives: The clinical significance of p53 supressor gene nucleoprotein immunostaining in ovarian cancer has not been determined.

Materials and Methods: p53-mut (Dako Inc., Denmark) expression was studied by immunocytochemistry from fresh frozen tissues in a series of 94 patients with ovarian cancer. The median follow-up time of the patients was 5 years.

Results: The 69 (73.4%) carcinomas stained positive for p53-mut expression. Positive staining for p53 expression was associated with clear cells and serous morphologic types, poor morphologic grade of differentiation, III-IV stages FIGO, DNA ploidy, but not with age and residual tumor size. Patients with III-IV stages FIGO with positive staining had only 18.5% 5-year survival compared with 87.5% survival rates in patients with p53 negative tumors, ($p < 0.05$). In a multivariate analysis, positive p53 staining was associated with poor survival together with residual tumor size less 2 cm.

Conclusions: Although p53-mut protein immunostaining is associated with several other prognostic factors in epithelial ovarian cancer, it may also have independent prognostic value in this deasease.

Disclosure of Interest: None declared.

P-067

Dysgerminoma: A Case Study of an 11-Year-Old Female

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Clinical Presentation: An eleven-year-old premenarchal girl presented with a history of worsening left loin pain for one week. On the day of presentation, the pain was associated with nausea and vomiting. A previous ultrasound (US) had revealed a left adnexal mass. The working diagnosis was suspected ovarian torsion. A subsequent US could not visualise either ovary but revealed mild hydronephrosis and a bulky uterus with irregular endometrial thickness. A possible cause for the uterine bulkiness was a malignancy. A laparoscopy was performed and the findings were haemoperitoneum with old blood seen in the pelvis and around the liver, a grossly enlarged torted left ovary which had a haemorrhagic and necrotic appearance, a suspected ruptured left ovarian cyst, and necrotic material which was favoured to be of ovarian origin. A small sized right ovary was suspected to be a streak gonad. The left necrotic ovarian cyst contents were sent for laboratory analysis. Cytology received 40 ml of moderately bloodstained necrotic material.

Cytological and Histological Findings: The smears were cellular and contained highly atypical cells occurring singly. The cells contained nuclei with granular chromatin, nucleoli and a scant amount of delicate and ill-defined cytoplasm. The appearances were highly atypical and the features favoured malignancy. Immunohistochemistry was performed on three pieces of cream tan tissue received by Histology. The tumour cells were positive for Oct 3/4, D240, focally positive for pan-keratin and focally weakly positive for CD117/c-kit. The tumour cells were negative for inhibin, calretinin, EMA, CD30, CD68, WT1, SMA, S100 and SOX 10.

Follow-Up Studies: Cytogenetic examination showed complete gonadal dysgenesis with 46, XY karyotype. Resection of the left adnexal mass showed a ruptured dysgerminoma and the right ovary was a streak gonad with gonadoblastoma.

Discussion: The features were most consistent with a germ cell tumour. The favoured diagnosis on morphology was dysgerminoma. The assessment was hampered by the limited sample and extensive necrosis. The differential diagnosis of a mixed germ cell component including yolk sac tumour could not be excluded. It is important to differentiate dysgerminoma from non-germ cell tumours, especially large cell lymphoma[1]. In this case, immunohistochemistry stained a minor population of mixed lymphocytes, positive for LCA, CD5 and CD20. Cysts and tumours of the ovary occur infrequently in children[2]. A review of 105 cases of dysgerminoma established a median age of 22 years, with a range from 6 to 56 years[3].

Disclosure of Interest: None declared.

P-068

A Case of Right Ovarian Cancer with Umbilical Metastasis

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Umbilical metastasis is considered to be 1–3% of all intra-abdominal and pelvic malignancies. Stomach, colon, rectum, ovary and other organs have been considered as the primary tumors. It was first reported in 1928 and has been called Sister Mary Joseph nodule (SMJN) since 1949. An umbilical metastasis is believed to be a sign of bad prognosis. However, recently, an improvement in the prognosis and a better survival rate had been reported with chemotherapy after surgery. Here, we present a case of right ovarian cancer with umbilical metastasis. (Case) A 76-year-old female complaining with an easily bleeding umbilical tumor came to our hospital for dermatology consult. She was diagnosed with teleangiectatic granuloma and received treatment with antibiotics. Since no improvement was observed, plastic surgery was consulted. A right ovarian cyst and pelvic disseminations were noticed by imaging studies and a high level of CA125 was observed. With the suspicion of a right ovarian cancer with umbilical metastasis, gynecology was consulted. Bilateral salpingo-oophorectomy, umbilical and omentum tumor resections were performed. During surgery, intra-abdominal metastatic lesions as large as a chicken egg were observed. Also, millet-seed sized lesions were noticed in the vesicouterine and douglas pouch. A cystic lesion of 2–3 cm approximately was observed in the right ovary. Small quantity of ascites was noticed and cytology examination was performed. Cytology findings: nuclear protrusion and mirror ball-like clusters were observed. Large tumor cells with markedly atypical nuclei were noticed. Resected specimen histology findings: In the umbilicus, ovary, and omentum, protuberant tumors were observed. The tumor cells showed villous proliferation and distinct nucleoli. Psammoma bodies were noticed in the ovarian tumor, which was the primary focus. As a result of the previous findings, serous papillary adenocarcinoma was diagnosed. Since not all the intra-abdominal and pelvic metastatic lesions were able to be resected, the patient is now in chemotherapy treatment and the CA-125 level is notoriously decreasing.

Disclosure of Interest: None declared.

P-069

Cytologic Features of AFP-Producing Ovarian Tumor in Ascites

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Background: Alpha fetoprotein (AFP)-producing ovarian tumor (APOT) is primarily seen in younger women although rarely reported in postmenopausal patients. Cytological examination of the ascites was done in a rare case of APOT in a perimenopausal woman.

Case: A woman in her 50s. Gravida 0 Para 0.

Clinical History: The patient presented with constipation in the other hospital. Abdominal ultrasonography demonstrated swelling of the right ovary. High values of cancer antigen 125 (287 U/ml) and AFP (145 ng/ml) were identified. She underwent total hysterectomy and bilateral salpingo-oophorectomy. Adjuvant chemotherapy was discontinued because of the side effects. Positron emission tomography-computed tomography scan revealed cancerous peritonitis one year after the operation. She was admitted to our hospital for treatment of recurrent ovarian tumor.

Cytologic Findings: Papanicolaou-stained smears revealed clusters of large round cells. The tumor cells had plump cytoplasm, large hyperchromatic nuclei, multiple prominent nucleoli, thin nuclear membrane, various sizes of vacuoles in cytoplasm, indistinctive cellular borders, and discohesive pattern. Remarkable cellular pleomorphism was shown. Some naked cells and hyaline globules were observed.

Histologic Findings: The right ovarian tumor contained some histological components. The tumor component which was macroscopically solid and pale yellow in color showed small-sized atypical glands with abundant fibrous stroma without hemorrhage or necrosis. The tumor cells were columnar and possessed the cytoplasmic vacuoles and cilia, suggesting that the component is adenofibroma or borderline tumor. Cystic tumor component with massive hemorrhage and necrosis was also present. The tumor component included histologic features such as macrocystic, microcystic or reticular, glandular, solid and endodermal patterns, suggesting that the component is yolk sac tumor. High mitotic activity and nuclear atypia indicated highly malignant potential.

Conclusion: Some previous reports emphasized the importance of a combined approach with cytology and immunocytochemistry when dealing with ascitic fluid materials from uncommon ovarian malignancy. In the present examination, we experienced that detailed observation of the cytological features leads to correct diagnosis of yolk sac tumor component in ascitic fluid specimen.

Disclosure of Interest: None declared.

P-070

Cytologic Aspects and Histopathologic Particularities in a Case of Bilateral Ovarian Serous Carcinoma

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Objectives: Cytological identification of malignant cells and histopatological and immunohistopatological confirmation in a malignant bilateral ovarian tumor in a 38 years old female admitted in depart of Gynecology in Emergency St. Pantelimon Hospital.

Clinic Aspects: giant pelviabdominal tumor, uterin subserous fibromas and cicatricial uterus after two cesareans operations. Diffuse abdominal pain predominant at inferior level. Slow intestinal transit. Loose of weight: 15 kg in 3 months.

Materials and Methods: Echographic aspects: Three tumors with mixt echostructure, pluriseptated, first tumor: 35/40/25 cm, second 12.9/8.7 cm and third in the middle 12.5/10 cm. Liquid in abdominal cavity. Right ureterohidronephrosis. No liquid in Douglas spaces.

CT aspects: three coalescent tumors 2 in left side and one in right latero-pelvin side, with polycyclic margins, with cystic areas with intraluminal projections, post-contrast aspects are nonomogen zones, mixt densities: solid and cystic arias. One tumor in left side 13.9/9 cm diameter, one in the middle with 12.5/10 cm and one in right with 12.9/8.7 cm. This 3 tumors look coalescent in CT images. In the median line is described the third circular and homogenous tumor. Infiltration of uterine wall cannot be appreciated. Pelvic microadenopathy is present.

Cytology from peritoneal liquid are prelevated.

Total hysterectomy with bilateral anexectomy, lombo-aortic lymphadenectomy and areas of epiplooic tissue are taken.

Histopathology and immunohistochemistry for confirmation of diagnosis.

Cytology from peritoneal liquid: Liquid centrifugation and Giemsa and Papanicolaou staining of slides.

Macroscopic Findings: Both ovaries with tumoral aspects with cystic and solid areas, necrosis and intaluminal projections in cystic spaces. Left ovarian tumor is biloculated and look a like two tumors in CT images. In the median line this tumors are coalescent. Right tuba is dilated. Lombo-aortic adenopathy and multiple epiplooic metastasis.

Results:

Cytopatologic Diagnosis: Serous adenocarcinoma cells.

Histopathology and Immunohistochemistry Confirmation: High grade bilateral ovarian serous carcinoma with epiplooic metastasis.

No lomboaortic metastases are found-only reactive aspects inclusive in immunohistochemistry: P16+, KI67++, CK7++, P53+++, ER–, PRG–.

Conclusion: Young women can develop aggressive giant ovarian tumors with epiplooic metastases in a short period of time.

Cytology is a good variant to discover malignant cells in peritoneal liquid before surgery by punction or laparoscopy or to use cytology for diagnosis as an extemporaneous cytology.

Disclosure of Interest: None declared.

P-071

Imprint Cytological Diagnosis of Metastatic Lymphnodes during Surgery in Gynecological Cancers

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Objectives: Cancerous lesion in Para-Aortic node (PAN) is the remote metastasis in uterine cervical Cancer. Our survey showed that PAN metastasis in cervical cancer must be suspected in 3 or more metastatic nodes in Pelvic nodes (PLN) or corpus extension, which can be detected during surgery. Our study aim is to validate the utility of the imprint cytology in diagnosis of metastatic lymph nodes during surgery, instead of intraoperative rapid pathological diagnosis.

Materials and Methods: When we found enlarged lymph nodes (more than 10 mm in size) on PLN during surgery (2011.4–2013.12), we submitted both the imprint cytological and pathological diagnosis in 27 uterine cervical cancer cases and 12 uterine corpus cancer cases and compare the results of both diagnosis and preoperative imaging studies.

Results: 1, Number of lymph nodes which was submitted to imprint cytology was 110. Among them, 39 lymph nodes (35.5%) had metastatic lesion. This diagnosis by imprint cytology was in agreement with one by pathological diagnosis for 91.8%.

2, The size of metastatic nodes and no metastatic nodes were 23.6 mm and 16 mm ($p < 0.05$), respectively.

3, The size of metastatic nodes and no metastatic nodes in squamous cell carcinoma of the uterine cervix were 24.2 mm and 15.5 mm ($p < 0.05$), respectively.

4, Comparison of the results of imprint cytology with ones of CT imaging showed that the specificity was similar (94.2% vs. 91.5%) and sensitivity in imprint cytology was better (87.2% vs. 51.3%).

5, We experienced 7 false positive and 2 false negative cases, which might be caused by technical problems during preparation of specimens.

Conclusion: Imprint cytological diagnosis of metastatic lymph nodes during surgery might be a useful tool instead of intraoperative rapid pathological diagnosis.

Disclosure of Interest: None declared.

Breast

P-072

Assessment of HER2 Status of Metastatic Breast Carcinoma on Cell Block Preparations of Fine Needle Aspirates Is Unreliable

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Objectives: HER2 status of breast carcinoma is a powerful prognostic and predictive biomarker-particularly in the metastatic setting. Limited data is available regarding assessment of HER2 on cell block preparations (CBP). The primary objective of this study was to assess correlation between HER2 results obtained via immunohistochemistry (IHC) and fluorescence in-situ hybridization (FISH) in cases of metastatic breast carcinoma (MBC) on CBP. Secondary objectives included study of inter-observer variability in interpretation of HER2 on IHC, and concordance between HER2 results on CBP and formalin-fixed paraffin embedded material (FFPEM).

Materials and Methods: Cases of MBC diagnosed on fine needle aspirates (FNA) with HER2 testing performed via IHC and FISH on CBP over 5 years (2010–2015) were reviewed. CBP material was fixed in an ethanol-based fixative (CytoRich Red Fixative system, BD). HER2 IHC was performed using polyclonal antibodies against Cerb-2 (Dako 0485). HER2 FISH testing was performed using the LSI HER2/neu/CEP17 probes (Vysis/Abbott Molecular Inc., Des Plaines, IL).

Results: Seventeen cases (all female, median age: 59) were analyzed. 41% of CBP were products of bone FNA (7/17). Other sites included lymph node (3), lung (2), pleural fluid (2), liver (1), skeletal muscle (1) and mesentery (1). Median interval between diagnosis of primary carcinoma and FNA of metastasis was 5 years (range: 10 months-32 years). FISH was inconclusive due to suboptimal specimen quality in 2 cases. Correlation between IHC and FISH results was as follows: IHC 0/1+ (0/2; 0% amplification), IHC 2+ (2/12; 16.7% amplification) and IHC 3+ (0/1; 0% amplification). Inter-observer agreement of IHC scoring between 2 pathologists who independently reviewed IHC slides was fair (66.7% agreement, $\kappa = 0.31$). Comparison of HER2 results on CBP with FFPEM (primary carcinoma or metastasis) showed a high discordance and slight agreement (discordance rate = 37.5%; $\kappa = 0.02$).

Conclusions: In this study**,** (a) 16.7% of MBC cases that scored 2+ on IHC showed amplification on FISH, (b) there was poor inter-observer agreement in HER2 scoring of IHC on CBP, and (c) there was high discordance between HER2 results obtained on CBP and FFPEM. Our results indicate that HER2 testing of MBC on CBP may be unreliable.

Disclosure of Interest: None declared.

P-073

Is FNA Still a Useful Tool in the Diagnosis of Breast Masses? A 5-Year Review with Cytohistologic Correlation in a Teaching Training Hospital in Bohol Island, Philippines

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Background: Breast cancer is the most common cancer among women worldwide. In the Philippine National Cancer registry, 1 in every 13 Filipino women is likely to suffer from breast cancer. Fine needle aspiration cytology (FNAC) is a reliable, fast, cost effective, safe and non-invasive technique practiced worldwide in breast cancer diagnosis.

Objective: To assess the value of FNAC as a rapid diagnostic tool in the local setting with the expectation to provide an immediate and highly reliable diagnosis in more than 90% of breast lesions.

Methods: From January 2010 to December 2014, palpation guided FNAC of the breast was performed on 1465 cases (1389 females and 76 males) at the GCGMH Department of Pathology. All FNAC were performed by Pathology residents, Pathologists and Cytopathologist. Smears were fixed in 95% ethyl alcohol and stained with Papanicolaou method. The FNAC smears were retrieved, reviewed blindly and reclassified into 5 categories (C1-C5). There were 306 aspirates with final histopathologic diagnoses obtained from core needle biopsy, incisional and excisional biopsy or mastectomy to give an assessment of the diagnostic performance of FNAC.

Results: The FNAC findings showed: 13 (4.25%) unsatisfactory cases (C1); 160 (52.29%) benign cases (C2); 23 (7.52%) atypical cases (C3); 9 (2.94%) suspicious cases (C4) and 101 (33.01%) malignant cases (C5). There were 120 (39.22%) histologically proven carcinomas and 186 (60.78%) benign lesions. In both the benign and carcinoma group, tumor diameters ranged from 0.8 to 10 cm. From the subsequent histopathologic diagnoses, 3.92% (12/306) with benign cytology turned out to be malignant (false negatives) and 0.65% (2/306) with malignant cytology turned out to be benign (false positives). The FNAC had 90% sensitivity, 99% specificity, 98% positive predictive value, 99% negative predictive value and 95% accuracy in this study. The risk of malignancy for each category is as follows: C1 = 15%; C2 = 4%; C3 = 13%; C4 = 78% and C5 = 100%.

Conclusion: Despite the increasing preference for core needle biopsy among surgeons, FNAC still continues to be an acceptable, affordable, quick and valuable tool contributing significantly to early breast cancer diagnosis and treatment, particularly in developing countries like the Philippines. Owing to its high sensitivity and specificity, it can be used as a screening and confirmatory diagnostic tool in breast cancer. Malignant and benign interpretations of breast FNAC give highly accurate prediction of outcomes but must be correlated with clinical and mammographic findings.

Disclosure of Interest: None declared.

P-074

Can Atypia in Breast FNA Be Tamed?

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Objective: To assess the inter-observer and intra-observer variability of statistically significant cytomorphological criteria commonly associated with atypical C3 category.

Material and Methods: Seven certified cytologists were given sixty C3 FNA cases to blindly rescreen searching for five statistically significant cytomorphological criteria commonly associated with C3 cases. The selected criteria were identified in a previous study by a robust statistical analysis from a set of 230 C3 cases. The selected criteria included myoepithelial cells or bare bipolar nuclei, cohesiveness, cystic background and the specific architectural features of papillary fragments or tubular structures. Two cytologist repeated the rescreen exercise 6 months later to obtain intra-observer data. Inter-observer and intra-observer agreement for each criteria was calculated using Kappa statistics.

Result: The inter-observer agreement was poor for all criteria except tubules which performed badly. This signifies much variation in the identification of the selected criteria between participating cytologists. One cytologist achieved moderate intra-observer agreement for all criteria except cohesion, whilst the second cytologist showed poor agreement for all criteria.

Conclusion: The clinical usefulness of FNA cytology of the breast is dependent upon the ability to reliably allocate samples into diagnostic categories, whereupon appropriate management strategies can be based. The well-defined cytological criteria for the diagnosis of benign and malignant lesions are more reliable than those for the indeterminate/atypical lesions. This study has shown the interpretation of cytomorphological criteria commonly associated with the C3 atypical category cannot be reliably reproduced. Inter-observer and intra-observer variability remains a significant challenge for cytologists. Lack of agreement on the diagnostic interpretation or equivocal criteria creates doubt and limits the clinical value of the result.

Disclosure of Interest: None declared.

P-075

FNA Findings in a Case of Superficial Thrombophlebitis of the Breast (Mondor's Disease)

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Objectives: Superficial thrombophlebitis of the breast (Mondor's disease, MD) is an infrequent, benign, self limiting disease characterized by thrombophlebitis of the superficial veins of the

chest wall. Although the diagnosis of MD is usually based on history and clinical examination findings, FNA could be indicated in cases with non typical presentation either clinically or on imaging studies.

Materials and Methods: We present a case of MD with non typical presentation in which FNA was performed.

Results: A 55 year old woman with no previous significant medical or surgical history presented with a strong pain and a palpable lump on her right breast on physical examination. Imaging studies followed. Mammography showed asymmetric density on the upper outer quadrant of the right breast and architectural distortion corresponding to the clinical findings. On US lobular hypoechoic lesions with anechoic, often tubular areas with only peripheral mild blood flow were seen. FNA of the palpable mass was decided in order to exclude malignancy. Cytology showed a heavy inflammatory infiltrate consisted of polymorphs and histocytes. Also, groups of spindle shaped cells with benign features probably of fibroblastic origin were observed. A few groups of degenerative epithelial cells were also seen. Cytology report was negative for malignancy, compatible with an inflammatory reaction and fibroblastic response. Patient was treated with anti-inflammatory drugs and antibiotics and after 4 weeks the lesion resolved completely.

Conclusion: Awareness of the disease is important for the cytopathologist in the diagnostic evaluation of the cytologic material. Cytologic findings reflect the histologic features described during the natural process of the disease.

Disclosure of Interest: None declared.

P-076

Occult Metastases in Sentinel Lymph Nodes among Breast Cancer Patients

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Objectives: The present study attempts to clarify the clinical significance of occult metastases in sentinel lymph nodes among breast cancer patients.

Materials and Methods: The subjects consisted of 1043 cases with clinically node-negative breast cancer who had undergone sentinel node biopsy in our hospital, between 2000–2010. After surgeries and also adjuvant therapies, interval clinical evaluations were performed.

Results: Postoperative pathological examination revealed 49 cases with occult metastases (26 isolated tumor cells and 23 micrometastases), despite negative intraoperative results. At a median follow-up of 75 months, eight cases with occult metastases developed recurrences. Axillary and distant relapse rates were 8.2% respectively, which were significantly higher than node-negative cases ($p < 0.001$). The relapse rates were not associated with the size of occult metastases. When the occult metastasized cases were treated with no further axillary surgery, the relapse rate was not increased compared with the cases who were diagnosed sentinel node involvements intraoperatively and received sequential axillary dissections.

Conclusions: However occult metastases in sentinel nodes have a little impact for axillary and distant relapse, sentinel lymph node biopsy alone with no further axillary surgery is an appropriate procedure for breast cancer patients with sentinel node occult metastases.

Disclosure of Interest: None declared.

P-077

Metastatic Breast Carcinoma in Plural Fluid: Correlation of Receptor and HER-2 Status with the Primary Carcinoma

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Objectives: Documenting the four molecular subtypes of breast carcinoma is significant as they determine response to therapy, disease free interval and survival. The aim of this study is to document the sub types defined by immunocytochemistry (ICC), expression of estrogen receptor (ER) or progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER 2): ER/PR+,HER2+; ER/PR+,HER2–; ER/PR–,HER2+; and ER/PR–,HER2– in metastatic breast carcinoma in pleural fluids and compare them with their expression in the primary tumor.

Materials and Methods: Over a period of 18 months, 13 cases of invasive breast carcinoma with metastases to the pleural cavity were studied for the subtypes. ER, PR and HER2 were determined by ICC in the primary breast tumor and the cell blocks of the pleural fluid with metastatic carcinoma.

Result: Age ranged from 33–75 years. The primary tumor ER/PR+,HER2+; ER/PR+,HER2–; ER/PR–,HER2+; and ER/PR–,HER2– were 4, 7, 1 and 1 respectively. In the cell blocks of the metastatic tumor in the pleural fluid the sub types ER/PR+,HER2–; ER/PR+,HER2+; ER/PR+,HER2–; ER/PR–,HER2+; and ER/PR–,HER2– were 5, 3, 3 and 2 respectively. There was complete correlation in 9 of the sub types in the primary and metastatic tumor. However, there were 3 cases where the metastases was positive for HER 2 and one case which became triple negative from ER/PR+,HER 2–.

Conclusion: Determining the molecular sub type in metastatic breast carcinoma is of importance as it greatly affects the management as 23% of our metastatic tumors became HER2 positive which would thus require anti HER 2 drugs.

Disclosure of Interest: None declared.

P-078

Non-Hematolymphoid Metastatic Neoplasms to the Breast of 10 Cases

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Objectives: The breast lesion metastasis form extra-mammary malignancy is rare with the incidence of 0.4–1.3% and most of them are hematolymphoid neoplasm. To evaluate the cytological features of metastatic extra-mammary tumors could provide the accurate diagnosis and prevent unnecessary surgery.

Materials and Methods: Ten cases of non-hematolymphoid metastatic neoplasms to the breast were identified in a series of 18211 cytological specimens of the breast at Koo Foundation Sun Yat-Sen Cancer Center from 2006 to 2015. These specimen types included core biopsy imprint smears (4 cases), fine needle aspiration with cell block (2 cases), and the fine needle aspiration only (4 cases). All of these 10 cases were confirmed by ancillary studies performed on histology specimens or FNA materials of the breast lesions, including immunostains and the testing of Epstein-Barr virus.

Results: The ten patients comprised eight women and two men, with a mean age of 49 years (range of 33 to 67 years). The primary sites were varied including the lung (5 cases), prostate (1 case), thymus (1 case), ovary (1 case), pancreas (1 case) and nasopharynx (1 case). Small cell lung cancer and thymic carcinoma were the two cases which present as breast lesions with unknown primary malignancy initially. The cytological patterns and immunostain results were not only different from the primary breast cancer but also similar to the primary site of the previous malignant lesions and raised the suspicion of extra-mammary solid tumor involving the breast lesions.

Conclusion: Metastasis to the breast from an extra-mammary neoplasm usually indicates disseminated metastatic disease and a poor prognosis. The presence of unusual cytomorphological patterns on breast specimens should alert the cytopathologists the suspicion of a metastatic breast neoplasm. To avoid misdiagnose the extra-mammary malignancy metastasis to the breast, compare the morphology with primary malignancy is highly recommended. A detailed history of the patients, clinical correlation, and ancillary studies such as immunohistochemical or immunocytological profiles are useful to establish an accurate diagnosis and provide appropriate managements for these patients.

Disclosure of Interest: None declared.

P-079

A Case Report of Breast Metastasis from Nasopharyngeal Carcinoma

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Objectives: To report the experience of diagnosis of breast malignancy metastasis from nasopharyngeal carcinoma by fine needle aspiration cytology. The prevalence of nasopharyngeal carcinoma is higher associated with some geographic areas and ethnic groups especially in Southeast Asia and Africa. The most common sites of metastatic nasopharyngeal carcinoma include cervical lymph nodes, bones, lungs and liver, but the breast lesion is extremely rare reported.

Material and Methods: A 42-year-old woman was diagnosed with undifferentiated nasopharyngeal carcinoma at stage T2N3aM0 in Jul 2012. Twenty months later, when she received the third course of combined chemotherapy and radiation for the treatment of liver and para-aortic lymph nodes metastasis, the bilateral breast masses and left axillary lymphadenopathy were found. According to the radiological findings, ultrasound guided FNA of right breast and core biopsy of left breast were performed to differentiate metastatic nasopharyngeal carcinoma from the primary breast cancer.

Result: The morphologic features of breast FNA, biopsy and previous nasopharyngeal biopsy are all similar. The tumor cells are characterized by moderate nuclear pleomorphism, hyperchromatic to vesicular chromatin with prominent nucleoli and indistinct cell border. Lymphoplasmocytic permeation to tumor cell clusters is noted. There are no in situ components found in the breast histology slide. The differential diagnosis should include metastatic nasopharyngeal carcinoma, and several types of primary breast cancer such as lymphoepithelioma-like carcinoma, medullary carcinoma and infiltrating ductal carcinoma or lobular carcinoma with inflammatory stroma. The Immunohistochemical stains of breast biopsy specimen are all negative including Cytokeratin 7, Estrogen receptor, Progesterone receptor, HER2 and GATA-3. According to the positive result of in situ hybridization for EBV-encoded mRNA (EBER) in both breast tissue and previous nasopharyngeal biopsy, the carcinoma cells of breast may be consistent with metastasis from nasopharyngeal carcinoma as primary site.

Conclusion: It is important to compare the unusual features with previous diagnosed neoplasm when the cytomorphological features are not matching the typical breast carcinoma. Early and accurate diagnosis of secondary breast involvement could provide appropriate management and avoid unnecessary harmful treatments in these patients.

Disclosure of Interest: None declared.

P-080

Cytopathological Study of 50 Cases with Solid-Papillary Carcinoma of the Breast

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Objective: Solid-papillary carcinoma (SPC) of the breast was first proposed as an uncommon category of the breast tumor by Maluf et al. in 1995. And it has been established for the first time as a tumor entity of the breast by WHO classification of 2012. The tumor is considered to account for <1% of the breast carcinomas. Most cases occur in postmenopausal women, with a mean age at presentation in the seventh decade of life. We report the clinical and cytopathological characteristics of 50 cases of the SPC.

Material and Methods: All the patients were Japanese women, aged 31–97 (mean age 64.6 yr). All the specimens were taken from the palpable masses by fine needle aspiration or nipple discharge. We made cytological, histological, and immunohistochemical examinations of all the cases.

Results: Cytologically, 28 cases showed high cellularity, highly discohesive clusters with numerous isolated cells. The tumor cells showed small round to oval and bland nuclei with finely granular chromatin in 47 cases and inconspicuous nucleoli in 49 cases. A low nuclear-cytoplasmic ratio and eccentric nuclei were seen in 42 cases. We have detected neither abnormal naked nuclei of tumor cell origin nor oval naked nuclei of myoepithelial cell origin. Histologically, all the tumors showed solid and expansive growth of epithelial tumor cells in the duct lobular units accompanied by delicate fibrovascular septa. Rosette and nuclear palisading at the stromal interphase were always seen. The tumor cells were positive for Grimelius staining and/or chromogranin A by immunohistochemistry in all the cases.

Conclusion: We concluded that the coexistence of malignant features such as large amount of tumor cells with discohesiveness and benign features such as the little nuclear pleomorphism was the most characteristic features for the cytology of SPC. The definite cytological diagnosis of SPC may require positivity on immunocytochemistry with neuroendocrine markers.

Disclosure of Interest: None declared.

P-081

Pitfalls in Cytological Diagnosis of Mucinous Carcinomas of Breast

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Introduction: Mucinous (colloid) carcinoma of the breast is relatively rare and accounts for 1–6% of all breast cancers. Traditionally, pure mucinous tumors and mixed infiltrating ductal carcinomas with a mucinous component have been described. Pure mucinous carcinoma of the breast has a favorable prognosis than the mixed variety noted in several studies. The common age of presentation is the postmenopausal group. Cytological diagnosis of these tumors is challenging.

Methods: A retrospective study was conducted at our institute. 22 cases consisting of both mixed and pure mucinous carcinomas diagnosed by cytology and verified on histopathology between Jan 2007 and Nov 2015 were included in the study. All cytology and histopathology slides were reviewed.

Results and Discussion: A total of 22 cases were evaluated. The age ranged from 34–78 years. Left breast was involved in 12 cases and the right breast in 10 cases. 19 cases were diagnosed as mucinous carcinoma on cytology. However 12 of these cases were reported as pure mucinous carcinoma on biopsy. All these cases displayed abundant mucin, small nuclei and/or regular nuclear outlines on cytology. 7 cases turned out to be mixed tumors on biopsy. Sparse mucin, large nuclei, irregular nuclear outlines or the presence of nucleoli were found in mixed mucinous carcinomas but not in pure tumors. Three cases were diagnosed as infiltrating ductal carcinoma (IDC) on cytology. Two of these turned out to be mixed carcinoma on histopathological examination. One of these patients had 2 nodules, diagnosed on histopathological examination as IDC and mucinous carcinoma. Out of the other two cases, 1 case was diagnosed as cellular variant of mucinous carcinoma and another case as mixed carcinoma on biopsy. Cellular variant of mucinous carcinoma can mimic low grade ductal carcinoma on cytology. A thorough examination of the slides for mucinous material and few thin capillary fragments provide a valuable diagnostic clue. Various other pitfalls are also discussed.

Conclusion: The distinction between pure and mixed mucinous carcinoma is important, because patients with the former type have a much favorable prognosis, with a low propensity for lymph node and distant metastasis. Owing to the various pitfalls, cytopathological identification of patients with pure mucinous carcinomas may be performed only in a limited number of cases.

Disclosure of Interest: None declared.

P-082

Cytological Characteristics of Poorly Differentiated Squamous Cell Carcinomas of the Breast

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Purpose: Squamous cell carcinoma (SCC) of the breast is very rare. Most of SCCs of the breast are triple negative and have aggressive clinical courses. Diagnosis using conventional cytological specimens is more likely to be missed when tumors are composed of poorly differentiated and non-keratinizing components. Distinguishing poorly differentiated SCC from invasive ductal carcinoma (IDC) and apocrine carcinoma (AC) is especially difficult. Therefore, we aimed to clarify useful cytological features for diagnosing poorly differentiated SCC of the breast.

Methods: We retrospectively evaluated 444 consecutive breast cancers which were resected between 2001 and 2013. We investigated cytological findings of poorly differentiated SCC (n = 9), and compared them with those of IDC (n = 15) and AC (n = 14). The following parameters were evaluated cytologically: streaming arrangement, nucleolar enlargement, dense nuclei, cannibalism, and necrotic background. To confirm histological features of SCC, breast cancers were also examined immunohistochemically using SCC markers.

Results: All of 9 cases of poorly differentiated SCC were confirmed to be positive for cytokeratin 5/6 and 34βE12. Cytological examination revealed the following characteristics in SCC cells: highly irregular shape, central hyperchromatic nucleus with coarse irregular chromatin, dense and basophilic cytoplasm, prominent nucleoli, and focal keratinization. These cells were predominantly present in streaming arrangement with necrotic background. Cytological specimens of SCC case showed significantly higher incidence of streaming arrangement ($p = 0.004$), necrotic background ($p = 0.003$) and nucleolar enlargement ($p = 0.03$) than those of IDC. Streaming arrangement ($p = 0.0008$) and cannibalism ($p = 0.0008$) in SCC were more frequent than in those of AC. Detection of two or more parameters described above in SCC showed higher sensitivity (78%) and specificity (93%) for correct diagnosis, in comparison with IDC. When differentiating from AC, the presence of parameter(s) in SCC improved sensitivity (80%) and specificity (100%).

Conclusions: These results suggest that cytological features such as streaming arrangement, necrotic background, nucleolar enlargement and cannibalism are useful predictors for diagnosis of SCC of the breast. We should pay more attention to these morphological findings in a daily practice.

Disclosure of Interest: None declared.

P-083

A Case of Solid Papillary Carcinoma in a Male Breast

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Solid papillary carcinoma-SPC is a rare histological type accounting for 1.7 percent of all breast carcinomas. We report a case of SPC arising in a male breast and describe its cytological findings.

Case Presentation: A 71 year old man first noticed a palpable mass of his left breast 1 year earlier. Initially there was no pain, but as the tumor increased, it became painful when in contact with clothing. When he came to the hospital, the size of the tumor was about 1 cm in diameter. Mobility of the tumor was good, accompanied by mild tenderness. The overlying skin was normal. Total resection of the left breast and the lymph node dissection were performed.

Cytological Findings: Imprint cytology of the needle biopsy specimen was done. Tumor cells we are observed as papillary clusters with scattered discohesive cells or individual cells. Internuclear distances were irregular in the cell cluster. Myoepithelial cells were not clear. Though not strictly polymorphic, nuclei were monotonous, some were small and some were large, with fine chromatin and slightly thick nuclear membranes. Irregular shaped nucleoli were prominent in some of the tumor cells. The cytoplasm was fine and granular.

Histological Findings: On gross examination, the tumor size was 1.5 x1.2 x 1 cm, whitish and solid. Microscopically, well defined nodules composed of low grade ductal cells separated by fibrovascular cores were seen. Most tumor cells were intraductal, though there were small invasive nests. Interstitial mucin were stained by alcian blue. Immunohistochemically, the tumor cells were positive for PgR, ER, EMA, AE1/AE3, and NSE, and negative for p63, CK5/6, CD10, and HER2. Few cells were positive for Chromogranin A and synaptophysin.

Conclusion: Although SPC is a rare carcinoma, if characteristic cytological findings were seen, we believe that it could be suspected even in a male breast.

Disclosure of Interest: None declared.

Respiratory Tract

P-084

C-MYB Over-Expression in Adenoid Cystic Carcinoma of Tracheobronchial Tree

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Objectives: Adenoid cystic carcinoma (AdCC) is a malignant epithelial neoplasm that occurs rarely in tracheobronchial tree (TBT). It has been seen that AdCC of various sites are associated with novel fusion transcript *MYB-NFIB* along with MYB protein overexpression. Expression of MYB protein in AdCC of TBT has not been evaluated previously.

Materials and Methods: Cases of AdCC of TBT diagnosed either on cytology or histology were included. c-Myb expression was analysed by immunocyto/histochemistry (ICC/IHC) (D2R4Y clone, 1:100 dilution, Cell Signalling Technology, USA). Clinicopathologic correlation was established.

Results: Twenty cases of AdCC of TBT were included in the study. Majority of samples were from lung (14/20, 70%), compared to trachea (4/20, 20%) and larynx (2/20, 10%). Out of the 4 cases diagnosed on cytology, three had corresponding histology specimens. The cytology samples included aspirates from subcarinal mass, bronchial wash, and bronchial brushing. Most of the patients presented with endobronchial mass. The age of patients ranged from 24 to 80 years with a mean age of 51.7 years. A male predominance was seen with a sex ratio of 1.6: 1. Two patients each underwent pneumonectomy and tracheal resection, while one patient underwent bronchoscopic debulking. ICC and IHC for c-Myb showed positivity in 10 out of 19 cases (52.6%). Follow-up was available in four cases, ranging from 2 months to 10 months (mean 6.7 month) and all the patients were alive at last follow up.

Conclusion: AdCC of TBT is rare and hence poses diagnostic difficulty. The presence of c-Myb immunopositivity in over half of these cases helps in differentiating it from its morphologic mimics and these cases may benefit from targeted therapy in future.

Disclosure of Interest: None declared.

P-085

Natural Helper Cells Mediate Respiratory Syncytial Virus-Induced Airway Inflammation by Producing Type 2 Cytokines in an IL-33-Dependent Manner

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in infants and young children. Increasing evidence demonstrates that type 2 cytokines including interleukin (IL)-4, IL-5 and IL-13 may be responsible for RSV-induced asthma-like symptoms and asthma exacerbations. It has been reported that natural helper cells, which are a non-T, non-B innate lymphoid cell type characterized by absence of lineage markers (Lin^-) and by expression of Sca-1, c-Kit and the IL-33 receptor ST2, produce abundant IL-13 during influenza A virus infection and contribute to airway hyperreactivity. However, little is known about pulmonary natural helper cells for the development of RSV-induced airway inflammation, particularly, the cytokine expression profile in natural helper cells during RSV infection. In this study, by using BALB/c mice that were infected intranasally with respiratory syncytial virus, it became clear that infection with RSV can increase not only the number of pulmonary natural helper cells but also the expression of mRNA for Th2-type cytokines in these cells, suggesting that natural helper cells may play a critical role in RSV-induced airway inflammation. In fact, adoptive transfer of pulmonary natural helper cells resulted in a significant peribronchial inflammation with a large number of mononuclear cells, in particular neutrophils, eosinophils as well as lymphocytes in lungs, following an augmented production of IL-4, IL-5 and IL-13 in the lungs of mice. In contrast, the amount of IFN-γ in the lungs of transferred mice was not affected markedly, suggesting that the protective immunity against RSV infection may not be influenced notably by adoptive transfer of lung natural helper cells. Indeed, viral growth and clearance were not altered significantly by adoptive transfer experiments. It should be noted that in vivo blockade of IL-33 can diminish not only the number of lung natural helper cells but also the expression of cytokine mRNAs, in particular the type 2 cytokine mRNAs in these cells, suggesting that the production of type 2 cytokines by pulmonary natural helper cells partly dependents upon IL-33. Taken together, our results demonstrate that pulmonary natural helper cells participate in RSV-induced airway inflammation by producing large amounts of type 2 cytokines in an IL-33-dependent manner.

Disclosure of Interest: None declared.

P-086

Immunohistological Analysis of EGFR, E-Cadherin and MMP2 Expression in Infiltrative Growth Pattern of Invasive Lung Adenocarcinoma

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Objectives: Histological subtypes of pulmonary adenocarcinoma are classified into lepidic, papillary, acinar, micropapillary and solid predominant by predominant growth pattern. It has been reported that lepidic predominant (LP) is higher frequency of lymph node metastasis and poorer prognosis than non-LP. In the progress of tumor, it is considered that overexpression of epidermal growth factor receptor (EGFR) is associated with tumor proliferation, and low-expression of E-cadherin, MMP2 by fibroblasts is associated with invasion and metastasis. However, there are few reports how these factors is related to progression of LP and non-LP. We report here relationship between immunohisto-

logical EGFR, E-cadherin and MMP2 expression and infiltrative growth pattern.

Materials and Methods: From 55 cases of lung adenocarcinoma, formalin-fixed paraffin-embedded sections were stained with anti EGFR antibody (31G7, Nichirei biosciences, Japan), anti E-cadherin antibody (HECD-1, Takara bio, Japan) and anti MMP2 antibody (42-5D11, Daiichi Fine Chemical, Japan). The number of cases of each histological subtype were as follows lepidic predominant: 19, papillary predominant: 16, acinar predominant: 16, micropapillary predominant: 0, solid predominant: 4 cases.

Result: EGFR overexpression were found in 6/19 (32%) cases of LP, 18/36 (50%) cases of non-LP, and MMP2 positive were 3/19 (16%) cases of LP, 14/36 (39%) cases of non-LP. These factors positive rate in non-LP was higher than LP. E-cadherin positive were 2/19 (11%) cases of LP, 6/36 (17%) of non-LP. E-cadherin positive rate was almost the same between LP and non-LP. For relationship between EGFR overexpression and T classification, EGFR overexpression were found in 3/8 (38%) cases of T1a, 1/6 (17%) cases of T1b, 2/5 (40%) cases of T2a in LP, and found in 2/6 (33%) cases of T1a, 4/7 (57%) cases of T1b, 11/18 (61%) cases of T2a, 1/4 (25%) cases of T2b, 0/1 (0%) cases of T3 in non-LP. EGFR overexpression rate was increased from T1a to T2a in non-LP. For relationship between MMP2 expression and lymph node metastasis, MMP2 positive were found in 3/17 (18%) cases of non-metastasis, 0/2 (0%) cases of metastasis in LP, and found in 9/26 (35%) cases of non-metastasis, 5 of 10 (50%) cases of metastasis in non-LP. In non-LP, MMP2 positive rate of metastasis group was higher than non-metastasis group.

Conclusion: It is suggested that EGFR and MMP2 expression is associated with infiltrative growth pattern both in LP and non-LP, and that EGFR overexpression may be related to tumor size increase, and MMP2 expression may be related to lymph node metastasis in non LP.

Disclosure of Interest: None declared.

P-087

Protective Effect of Acacetin on Lipopolysaccharide-Induced Acute Lung Injury via Reduction of P38 MAPK and JNK Phosphorylation

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Objectives: Acute lung injury (ALI), the serious and acute pulmonary inflammatory disorder, remains the high incidence and mortality in patients. Up to now, there are no effective therapy strategies available clinically for the improvement of ALI. Acacetin, belonging to the family of flavonoids, is present in a vast of plants, such as *Saussurea involucrate, Cirisium rhinoceros* Nakai, *Clerodendrum* inerme (L.) Gaertn, *Compositae*. Acacetin has been shown to cause beneficial effects against inflammation-related diseases such as inflammatory pain, Parkinson's disease, asthma, and cancer. The aim of this study to investigate the potential protective effects of acacetin and the molecular mechanisms involved in lipopolysaccharide (LPS)-induced ALI.

Materials and Methods: In the mice model, ALI was induced by intratracheal administration of LPS, and acacetin at various concentrations was intraperitoneal administration for 30 min prior to LPS treatment. Histopathological analysis and leukocytes infiltration were measured by hematoxylin-eosin method and Giemsa stain, repectively. Enzymes activities and lipid peroxidation were determined by commercially assay kits. Expression of cytokines and proteins was measured by ELISA and western blot.

Results: Pretreatment with acacetin inhibited histolopatholgical changes and leukocytes infiltration in lungs in LPS-induced ALI. Decreased activities of superoxide dismutase, catalase, and glutathione peroxidase induced by LPS were reversed by acacetin. Phosphorylation of NF-κB and degradation of IκBa were inhibited by acacetin in LPS-induced ALI.

Conclusion: In conclusion, the protective mechanisms of acacetin are up-regulation of antioxidative enzymes and inhibition of NFκB phosphorylation in LPS-induced ALI.

Disclosure of Interest: None declared.

P-088

Use of Endobronchial Ultrasound (EBUS) for the Evaluation of Lung Cancer Using Rapid on Site Evaluation (ROSE) by Senior Cytology Scientists – Experience in a Regional Laboratory Over a 16 Month Period

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Introduction: EBUS has recently become a very important tool to biopsy mediastinal lymphadenopathy for the staging of primary lung cancer, as well as identifying granulomatous responses in lymph nodes.

This study evaluates the EBUS results within a 16 month period. The laboratory examines approximately 1900 non-gynaecological specimens per year, and 25% of the fine-needle aspirates (FNA) are for EBUS evaluation.

Methods: All cases were attended by one of two cytologists with extensive experience in ROSE. Most cases were performed by one respiratory physician. Each EBUS pass has one fixed and one air-dried slide prepared, and the needle rinsed out in saline. After evaluation by a cytologist, it is decided if extra passes are required for further slide evaluation, cell block by FAA method for immunohistochemistry and/or genetic (e.g. EGFR) testing, flow cytometry or microbiology. TTF-1, napsinA, CK5/6 and p63 are the first

antibodies performed. If a neuroendocrine tumour is suspected, Chromogranin, Synaptophysin, CD56 and Ki67 are then performed.

Results: Of the 75 sites aspirated (52 patients), the overall adequacy rate was 92%, and the diagnosis of malignancy was achieved in 28 aspirates (38%). Forty aspirates (54%) were negative for malignancy, including 7 aspirates (9%) with granulomas consistent with sarcoidosis. One aspirate (1%) was suspicious and 6 aspirates (8%) were deemed inadequate samples. The malignant cases were classified as follows: primary adenocarcinoma of lung (13) aspirates, squamous cell carcinoma (6), small cell neuroendocrine carcinoma (5), large cell neuroendocrine carcinoma (1), lymphoma (1), poorly differentiated carcinoma (1), and secondary carcinoma (1). EGFR and ALK tests were performed in 7 and 9 primary lung adenocarcinomas, respectively. In the EGFR group, 2 aspirates were positive and 5 were negative; 6 aspirates were inadequate for testing. In the ALK group, all 8 aspirates tested were negative and 1 was equivocal. Three aspirates were inadequate for testing, and 1 was cancelled.

Conclusion: EBUS is a reliable method for the staging of lung cancer. This study emphasises the importance of cytology in the diagnosis and staging of lung cancer, and its ability to further categorise tumours using immunohistochemistry and molecular tests to guide appropriate treatment. ROSE attendance by cytologists increases the yield of material, allowing triaging and tests to be performed on the limited material obtained. These results compare favourably with experiences from those found in the literature.

Disclosure of Interest: None declared.

P-089

Evaluation of an Endobronchial Ultrasound (EBUS) Cytology Service in the Management of Suspected Lung Carcinoma

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Objectives: To evaluate the effectiveness of introducing an EBUS cytology service at our Institute on the diagnostic work up and staging for suspected lung cancer and other mediastinal pathologies.

Materials and Methods: All EBUS cytology samples reported at our Institute over a 9 month period (June 2014 to Feb 2015) were evaluated for turn-around time, processing protocol, sample adequacy, diagnostic categories, workload, and any requirement for further diagnostic workup.

Cohort size = 101; reporting cytopathologists = 7; data extracted by SNOMED search and from data maintained on an EXCEL spreadsheet.

Results: Data expressed as mean and (range) where necessary.

Number of subjects undergoing EBUS = 11.2 (8–17)/month.

Total number of pots received over study period for all patients = 297.

Number of pots received/patient = 2.9 (1–6).

Turn around time from receipt of sample to authorisation = 5.5 (2–23) days.

Cell-blocks from syringe washes revealed paucicellularity, did not improve diagnostic yield and increased processing, screening and reporting time.

Adequacy of samples: <50% of samples were adequate based on criteria of >40 lymphocytes/HPF & anthracotic pigment laden macrophages. 96% of samples were considered adequate based on modified criteria permitting adequacy in the presence of microbiopsies in the sample.

Diagnostic categories: Inadequate = 3, No malignant cells = 43, Suspicious = 7, Malignant = 39, granuloma = 9.

Subtyping of malignant and suspicious samples: Non-small cell carcinoma NOS = 3, squamous cell carcinoma = 8, adenocarcinoma = 15, lymphoma = 1, small cell carcinoma = 7, suspicious = 4, probable mesothelioma = 1, others = 7.

Immunocytochemistry (ICC) was performed on 45.45% of the test samples and molecular tests (EGFR & ALK) were done in 16.16% of the malignant samples.

Conclusions: The turn around times for the majority of the samples was between 2 to 11 days suggesting an effective service provision.

Syringe washes did not provide any additional information. Since evaluation samples of aspirate and syringe contents are being submitted in a single pot, thereby reducing workload. Modified criteria for assessing adequacy, by considering the presence of microbiopsies as a reportable sample, resulted in more samples being diagnostic and thereby avoided repeat EBUS. The histological diagnoses obtained from EBUS samples at our centre were marginally different compared to the data published from the local population treated at a neighbouring centre. The samples were adequate for ICC and molecular testing, allowing subtyping of the malignancy and aiding in the decision regarding the best treatment option.

Disclosure of Interest: None declared.

P-090

Combined (Mixed) Small Cell Neuroendocrine Carcinoma Admixed with Large Cell Neuroendocrine Carcinoma and Adenocarcinoma (SCLS and NSCLS) Diagnosed by Endobronchial Ultrasound-Guided Transbronchial Fine Needle Aspiration Performed from Paratracheal Mass

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Objectives: Endobronchial ultrasound-guided transbronchial fine needle aspiration (EBUS-FNA) is minimally invasive method of diagnosis from lymph nodes, as well as, solid lesions in the mediastinum. EBUS-FNA is using for the staging of lung cancer in patient with an established diagnosis of non-small cell lung cancer, or for diagnosis of a suspicious mediastinal lesion in patient with suspected lung cancer.

Materials and Methods: A 52 year old man with right upper peripheral paratracheal mass was diagnosed by computer tomography. FNA-EBUS was performed from the mass. Papanicolaou smears and cell block were prepared.

Results: Groups of small and large malignant cells with neuroendocrine features were found on a background of lymphocytes in the smears and in cell block. Immunostain results: CK7, CK8/18 (CAM 5.2), TTF1 and synaptophysin were positive in most malignant cells. Napsin-A was positive in a part of malignant cells. Ki67 was positive in 60% of malignant cells. LCA (CD45) was positive in lymphocytes (negative in tumor cells), and negative markers were p63 and chromogranin. Cytology diagnosis was combined SCLC AND NSCLC.

Conclusion: It is possible to diagnose combined SCLC AND NSCLC by cytology (EBUS-FNA), according to immunostains.

Disclosure of Interest: None declared.

P-091

Large Cell Neuroendocrine Carcinoma of Lung: A Rare Tumor

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Objective: To report cytological findings of a large cell neuroendocrine carcinoma of lung (LCNELC) which is a very rare neoplasm with poor prognosis.

Materials and Methods: CT-guided FNA findings of a LCNELC were reported with clinical, radiological, histological, and immunohistochemical correlation.

Results: A 73 year-old Thai male; a veteran and former smoker 40 pack-years with 10-year duration of smoking cessation, presented with fever and cough for 2 weeks. A month prior to the admission, he had suffered from chronic productive cough of white mucus, with no fever or loss of appetite. Two years ago, a lung mass in right upper lobe (RUL) had been found with clinical impression of pulmonary infection, but no further management at that time due to loss to follow-up of patient. CT-lung revealed a 6 cm mass in the RUL. CT-guided FNA of the lung mass showed hypercellular smear consisting of clusters of atypical cells with salt-and-pepper-like chromatin, prominent nucleoli, and focally abundant cytoplasm. Their nuclear sizes were approximately 4–6 times of that of a small lymphocyte. Focal nuclear molding, crushing artifact and some dispersed cells with necrotic debris were also observed. Cytologic diagnosis was positive for malignancy; suspicious of neuroendocrine carcinoma. Histologic findings of the CT-guided biopsy specimen revealed tumor cells arranging in trabecular, organoid, and cribriform patterns with tumor nuclear size more than 3 times diameter of a small resting lymphocyte; with prominent nucleoli; moderate to abundant cytoplasm; and extensive necrosis. Immunohistochemical studies were: synaptophysin (+; diffuse; strong intensity), chromogranin A (+; 50% of cells; weak intensity), NSE (+; <10% of cells; weak intensity), AE1/AE3 (+; focal), Ki-67 labeling index = 90%, and TTF-1 (+; diffuse; strong intensity); thus the diagnosis of large cell neuroendocrine carcinoma of lung origin was confirmed. Subsequent RUL lobectomy specimen revealed a peripheral tumor with similar morphologic appearance, focal rosette formation, and locoregional lymph node metastases.

Conclusion: Cytological findings from CT-guided FNA of a LCNELC with clinical, radiological, histological, and immunohistochemical correlation were reported. Key features to differentiate LCNELC from small cell neuroendocrine carcinoma are the significantly larger nuclear size, significant amount of cytoplasm and the prominent nucleoli.

Disclosure of Interest: None declared.

P-092

Cyto-Morphological Features of Chest CT Negative Early Lung Squamous Cell Carcinoma Screened by Sputum Cytology

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Background: In order to detect hilar type early lung cancer, sputum cytology has been performed for high risk candidate in Japan. But it is uncommon to identify preinvasive or micro-invasive lung carcinoma with sputum cytology. Here we show cases of chest CT negative, early lung squamous cell carcinoma screened by sputum cytology and analyzed their cyto-morphological features.

Methods: During 2007–2014, 73082 participants were screened to detect lung carcinoma and 80 lung cancers were detected with sputum cytology in our institute. Among them, eight cases were chest CT negative early lung cancer. With these cases, cyto-morphological features of atypical squamous cells (ASC) in the collected sputum smear were analyzed.

Results: In the eight cases, six were carcinoma in situ and two were micro invasive squamous cell carcinomas. All of them were male, mean age was 68.8 yrs old and mean smoking index was 60.0 pack/yr. Cytological diagnoses at the time of initial screenings were two as malignant, five as severe and one as moderate ASC. Mean follow up time between cytological identification and clinical diagnosis was 2.5 months. Of the screened ASC, predominant size was small in 6 cases (75%), and severe atypical squamous cells were few. Degenerated ASC were also relatively frequent. In all cases, large ASC were few and variation of size was limited.

Conclusion: In order to detect chest X ray negative early lung squamous cell carcinoma, screening of small size ASC was important. Screening of degenerated ASC and bright orangeophilic ASC with inconspicuous nuclear chromatin were also indispensable.

Disclosure of Interest: None declared.

P-093

Speedy Preparation and Use of LBC Specimens of Sputa by Cytorich Red

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Objectives: We have previously described an overnigh method (ONM) of preparing Liquid-based Cytology (LBC) specimens of sputa by Cytorich Red (CR; BD Diagnostics, Franklin, NJ, USA), their use and special staining. The background of such specimens tends to be clearer than that obtained by the direct smear (DS) method, and malignant cells are more easily detectable in LBC specimens than in DS ones ($p = 0.054$, n = 5). In the present study, we added cases and describe a method of preparing LBC specimens speedily with a shorter turnaround time (TAT).

Materials and Methods: In addition to the previous 79 samples, 22 and 13 sputum samples were examined at Nara Medical University Hospital and Medical Nara, respectively, between November and December of 2015. CR including 0.5% mucolytic agent ((±)-dithiothreitol, DTT; WAKO, Japan) was added to the sputa remaining after the preparation of DS specimens. The sputa were then fixed for 2 hours, agitated with the use of a Syringing Pipette (BD Diagnostics), fixed for 2 hours, centrifuged at 2000 rpm for 10 minutes and decanted, washed in distilled water and agitated with a syringe. The procedure was carried out according to the protocol recommended by BD (4 hours method, 4HM).

Result: In the additional study on ONM, mucus floated solids (MFS) arose in abundance on samples of great viscosity. On the other hand, the viscosity of 19 samples prepared by 4HM liquefied in a shorter period of time and we were able to produce LBC specimens without MFS. The quality of specimens prepared by 4HM was equal to or superior to that by ONM. Moreover, TAT by 4HM (mean = 11 hours) was shorter than that by ONM (mean = 56 hours) ($p < 0.005$, n = 35).

Conclusion: Sputa are known to contain some mucosal components. Assumably, MFS arose on the ONM specimens because only a mucolytic agent did not fully liquefy them overnight. Nonetheless, physically liquefying mucosal components by pipetting made possible the preparation of LBC specimens in a shorter period of time. We therefore consider that both chemical and physical means are needed for preparing LBC specimens of sputa. The 4HM may provide good quality specimens of sputa and a reduction in TAT. 0744-22-3051 (extension 4303).

Disclosure of Interest: None declared.

P-094

Fine Needle Aspiration Cytology of Lung Lesions and Correlation with Histopathology

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Introduction: Lung cancer is the leading cause of death worldwide. High mortality rates makes early diagnosis and treatment of utmost importance. Cytology plays an important role in the initial evaluation and diagnosis of these patients with various sampling techniques which include exfoliative, abrasive cytology and fine needle aspiration cytology (FNAC).

Aim: To assess the role of CT/USG guided FNAC of lung and pleural lesions and correlate the findings with biopsy.

Materials and Methods: 82 patients with lung lesions were evaluated with USG/CT guided FNA and biopsy between January 2011 & July 2012. The findings of FNAC were correlated with the biopsy wherever available, in order to assess the reliability of a cytologic diagnosis of the various lung lesions on FNAC. 5 cases with pleural lesions were also evaluated.

Results and Discussion: On evaluation of patients, the age ranged from 19–84 ys and a male preodominance (81.7%) was noted. Benign, suspicious and malignant lesions accounted for 8.5%, 3.7% and 87.8% respectively. On classification, adenocarcinomas accounted for majority of the malignant lesions (34.1%) followed by squamous cell carcinomas (26.8%). 57 out of 82 patients underwent biopsy of the lung lesions. When the FNAC findings were correlated with the histopathological biopsy findings, 6.7% (4) of cases did not correlate. Among these four cases, all were malignant lesions. However an accurate diagnosis of either adenocarcinoma or squamous cell carcinoma could not be rendered and thus a histopathological correlation was requested. Cytology helped to categorise the lesions as malignant, but however due to certain pitfalls in differentiationg adenocarcinoma and squamous cell carcinoma, an accurate diagnosis was not possible. Of the five pleural lesions evaluated, 4 were metastatic carcinomas which correlated on FNA and biopsy. One lesion was inflammatory. Thus the sensitivity and specificity of the study were 92.9% and 98.2% respectively. Carcinomas of high nuclear grade with prominent nucleoli, including poorly differentiated Squamous cell carcinoma and large cell neuroendocrine carcinoma can pose difficulty in subclassification. Differentiating adenocarcinoma and squamous cell carcinomas could also be challenging at times.

Conclusion: CT guided FNAC has emerged as a less invasive, cost effective, rapid and fairly accurate diagnostic aid in the evaluation of lung lesions. Correlation of the cytomorphologic features with the clinical features and radiologic imaging are absolutely critical in the accurate interpretations of respiratory cytology specimens.

Disclosure of Interest: None declared.

P-095

The Value of the Cell Block Based on the Fine Needle Aspiration Samples in the Lung Cancer Diagnosis

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Objectives: Immunocytochemistry played an important role in differential diagnosis of lung cancer subtype, according to the 2015 WHO classification of lung cancer. In order to improve the accuracy of the cytological diagnosis, we investigated cell blocks of cytological samples to distinguish benign cells from malignant cells and further subtype diagnosis by immunocytochemistry (ICC).

Materials and Methods: We collected 526 cases of computed tomography (CT)-guided percutaneous lung fine needle aspiration cytology cases in Shanghai Pulmonary Hospital during May to October in 2015. The diagnosis was carried out according to the 2015 WHO classification criteria of lung cancer. ALK were tested by BenchMark XT Staining Instrument with rabbit monoclonal antibodies (Ventana, clones D5F3).

Results: We found 59 cases with no cellular components after embedding. The success rate of cell block embedding was 88.78%. There was no significant difference in the success rate of cell block between the positive specimens, the suspicious of carcinoma specimens and the negative specimens. 32 (6.08%) suspicious of carcinoma cases were reduced to 10 (1.90%) after cell blocks were made. 417 (79.28%) positive cases were risen to 444 (84.41%, $P < 0.05$). 161 (30.61%) non-small-cell lung carcinoma were reduced to 33 (6.27%, $P < 0.05$) cases. And we diagnosed 8 (1.52%, $P < 0.05$) metastatic carcinoma of lung according to the cell block and ICC. 3 (3.85%) cases were ALK positive among 78 adenocarcinoma specimens.

Conclusions: Cell block and ICC are useful for diagnosis of advanced lung cancer. We can also use cell blocks to detect the ALK gene changes in the lung cancer patients. It is valuable to individualized treatment and evaluation of prognosis. Cell block is an effective complement for the histologic biopsy.

Disclosure of Interest: None declared.

P-096

Cancelled

P-097

Comparison of Cytologic Features in Bronchial Brushing, Washing and Sputum Specimens for Lung Cancer

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Objectives: In contrast with past years, when such subtyping in lung cancers had no therapeutic relevance, differentiating between adenocarcinoma (AC) and squamous cell carcinoma (SCC) is now important because new therapies have been developed that have different therapeutic or adverse effects depending on the histologic type. On the other hand, cytological appearances of lung carcinoma vary in different types of specimen, and we compared cytological findings from sputum, bronchial brushing and washing specimens.

Materials and Methods: Cases were selected in which all of the sputum, brushing and washing specimens were available in the same patients: 27 SCCs, 15 ADs, and 9 small cell carcinomas. Cytological features were systematically analyzed: 1) number, size and degree of overlapping of cell clusters; 2) size, shape and hyperchromaticity of nucleus; 3) thickness of nuclear membrane and distribution of chromatin granules; 4) conspicuousness of nucleoli; 5) thickness of cytoplasm; and 6) artificially-crushed nuclei. In addition, we measured the nuclear area and roundness by Image Pro PLUS (Media Cybernetics Inc.).

Results: Number, size and overlapping of cell clusters tended to be greater in brushing, washing and sputum specimens in this order in any histological types. In SCC, malignant cells of sputum specimen were more pyknotic, and showed greater angularity and irregular nuclei, coarse chromatin, thick nuclear membrane, unclear nucleoli, thick cytoplasm, and clear cell border than brushing specimens. In image karyometric analysis, brushing specimens showed larger nuclear areas and rounder nuclei than those of sputum specimens ($P < 0.01$). However, in AD, no difference of findings in the nucleus and cytoplasm was clear among three types of specimens.

Conclusion: In brushing specimens, cells usually appear adequate and sensitivity is generally higher than in sputum specimens. However, the decision of histological type is sometimes difficult in brushing specimens, because SCC and AD lack cytological degenerative changes that may help their differential diagnosis. In sputum specimens, it often occurs that we make a negative diagnosis because carcinoma cells are not apparent and we can't make a positive diagnosis because there are only a very few carcinoma cells with conspicuous degeneration. However, if the carcinoma cells appear adequate, the decision of histological type is easiest in sputum specimens. It is important to understand the cytologic characteristics of various cell types in each specimen.

Disclosure of Interest: None declared.

P-098

Epithelial-Myoepithelial Carcinoma of the Lung

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Background: Epithelial-myoepithelial carcinoma of the lung is an extremely rare low-grade carcinoma showing endobronchial localization. Cytological diagnosis of this tumor is difficult because of its rarity.

Patient: A 64-year-old male presented with an asymptomatic right pulmonary nodule detected at annual checkup. A 56 X 24 mm well-circumscribed tumor was observed in the right upper lobe. Bronchoscopic examination showed a polypoid tumor in the right B2 bronchus, which was diagnosed as adenoid cystic carcinoma by bronchial biopsy. Right upper lobectomy was performed.

Cytology: Brushing specimens showed mucous-like material surrounded by small oval cells with hyperchromasia and sheet-like cell cluster with high N/C ratio, irregular shaped nuclei, and prominent small nucleoli. The cytological diagnosis was mucoepidermoid carcinoma.

Histopathology: The tumor was composed of glands with double cell layer. Inner layer cells were eosinophilic with bland nuclei and outer cells had clear cytoplasms. Immunohistochemical staining showed positive for SMA and calponin in outer cells and positive for CKMNF116 in inner cells. Mitosis and necrosis were not observed. There were no metastases in the dissected lymph nodes. EGFR was wild type and ALK was negative. Pathological stage was IB due to T2aN0M0.

Conclusion: When making a diagnosis of a polypoid tumor arising from an endobronchial lesion, epithelial-myoepithelial carcinoma should be considered. Cytologically, existence of mucous-like structure resembling adenoid cystic carcinoma and two-cell pattern is useful for definitive diagnosis.

Disclosure of Interest: None declared.

P-099

Cytological Findings of Primary Pulmonary Meningioma with Immunohistochemical Examination: A Case Report

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Background: Ectopic meningioma that can occur in various organs except for central nervous system is very rare, but there are some reports of skin, nasal sinus, mediastinum and the other site's origin. Here, we report a case of primary pulmonary meningioma (PPM) with particular reference to cytological findings.

Case: A healthy 7th decade female visited our hospital for detail evaluation of a pulmonary nodular lesion in the right lower lobe, measuring 20 mm in diameter on a chest X-ray that was pointed out by routine medical examination. A transbronchial fine needle aspiration cytological examination performed. She received segmentectomy for the lung lesion then followed by thyroidectomy for a thyroid papillary carcinoma that was detected incidentally. She had no history of other tumor and no tumor detected in the central nervous system on imaging examination including CT and MRI after the operation. She has been well for six months after lung operation.

Cytological Findings: The cytological preparation showed clusters composed of spindle cells with whorl formations and psammoma bodies on the clear background. Many tumor cells were spindle-shaped with eosinophilic cytoplasm and small fusiform or round nuclei. The nuclei of tumor cells showed fine chromatin pattern and numerous intranuclear cytoplasmic inclusions. Immunohistochemical examination using imprint cytological specimen of resected lung revealed that tumor cells were positive for progesterone receptor (PgR), and negative for thyroid transcription factor-1 (TTF-1) and p40.

Histological Findings: Histologically, the tumor was diagnosed as meningioma, which showed similar findings of cytology. Immunohistochemically, the tumor cells showed positive for epithelial membranous antigen (EMA) and PgR, negative for TTF-1 and p40.

Conclusion: Although PPM is a very rare disease, it would be possible to include a differential diagnosis due to the characteristic cytologic findings described above. The combination of pathological and imaging examination can lead the correct final diagnosis.

Disclosure of Interest: None declared.

P-100

Cytological Findings Mimicking Cytological Malignancy, of Ciliated Muconodular Papillary Tumors/Mixed Squamous Cell and Glandular Papilloma of the Lung. Report of 4 Cases Including Cytological Review of 2 Cases

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Objectives: Ciliated muconodular papillary tumor (CMPT) of the lung is a rare benign tumor, which has been described firstly by Ishikawa (2002 in Japanese, Byori to Rinsho 2002;20:964–965), and precisely described by Kamata et al. (Am J Surg Pathol 2015;39:753–760). A case of mixed squamous cell and glandular papilloma (MSGP) was reported by Kozu et al. (Ann Thorac Cardiovasc Surg. 2014;20 Suppl:625–8, case 1). These two kinds of tumors have common histological features reflecting cytological findings. We report the cytological findings of 2 cases and compare their atypia to histological findings.

Materials and Methods: We experienced 4 cases of these tumors in 4 years in Shizuoka Cancer Center, of which two cases were examined cytologically pre- or intra-operatively. In all 4 cases (60–77 Y.O., mean 66.5 Y.O., M:F = 3:1, R:L = 2:2), the lesions were resected and histologically examined.

Cytological Findings: Two of 4 cases (cases 1 and 4) showed cytological atypia with nuclear and nucleolar enlargement, and necrotic background. These findings seemed to be suggestive of carcinoma pre- or intra-operatively.

Histological Findings: The tumors showed papillary or nesting proliferation of ciliated or non-ciliated columnar epithelia, squamous epithelia, and partially mucin-containing columnar cells in alveolar spaces or bronchial lumina. In general, these epithelial proliferating lesions showed little cellular or structural atypical morphology suggestive of malignancy, although there were locally degenerated or necrotic epithelial cells and/or reactive cellular atypia.

Discussion and Conclusion: In the cases which suggest possibility of CMPT/MSGP of the lung, even if the cytological findings might suggest malignancy, it should be kept in mind that the cytological atypia could be pitfalls for cytological diagnosis of CMPT/MSGP, benign tumors.

Disclosure of Interest: None declared.

P-101
Cancelled

P-102
A Case of Lung Metastasis of Ovarian Clear Cell Adenocarcinoma Showing Primary Lung Adenocarcinoma-Like Appearance

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Metastatic lung carcinoma rarely shows histological lepidic growth on alveolar walls which is similar to primary lung cancer. We describe a case of lung metastasis of ovarian cancer showing primary lung adenocarcinoma-like histology and imprint cytology was helpful in differential diagnosis.

Case Presentation: A 70-year-old woman, who had a history of hysterectomy and bilateral adnexectomy for ovarian clear cell adenocarcinoma performed 3 years before, and partial pancreatectomy for pancreatic tubular adenocarcinoma performed 4 years before, developed multiple ground-glass opacities in right lower lobe of the lung by follow-up CT scan. The radiological image was undeterminable whether primary lung cancer with its metastasis or multiple metastasis from other primaries. Video-assisted thoracoscopic partial resection of the lung was performed to determine the diagnosis.

Gross Finding: An 8x8 mm sized ill-defined whitish tumor with central anthracosis was seen on cut surface of the lung tissue.

Histological Findings: Dome-shaped cells with hyperchromatic nuclei proliferated along alveolar walls, resembling primary lepidic adenocarcinoma of lung. Fibrosis including collapsed alveoli was seen in central part of the tumor. It was particularly difficult to determine whether primary lung cancer or metastatic tumor by the intraoperative frozen section.

Cytological Findings: Loosely connected atypical cell clusters were seen on the imprint cytology specimens. Each cell had wide and clear cytoplasm and hyperchromatic nuclei, and sometimes showed hobnail-shaped appearanace. The cyological findings were very similar to those of ovarian clear cell adenocarcinoma.

Immunohistochemical Findings: The tumor cells were immunohistochemically positive for cytoketain 5/6, 34betaE12, and vimentin, negative for TTF-1. These immunohistochemical characteristics are consistent with ovarian clear cell adenocarcinoma rather than primary lung adenocarcinoma.

Conclusion: Imprint cytology often shows morphological characteristics of the tumor cells better than frozen histological section. Concomitant use of imprint cytology is effective to make correct diagnosis on intraoperative frozen section.

Disclosure of Interest: None declared.

P-103
Imprint Cytological Study of 3 Cases of Sclerosing Pneumocytoma of the Lung

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Objectives: Sclerosing pneumocytoma (SP) is a benign pulmonary lesion that frequently occurs in middle-aged woman and is incidentally found. The are not so many reports on cytological findings on pulmonary SP. In this study, I investigated imprint cytological findings of pulmonary SP.

Materials and Methods: During January 2009 and December 2015 in Departmwent of Diagnostic Pathology, Kochi Red Cross Hospital, we selected 3 cases of SP that both surgical resected and imprint cytology specimens have been obtained.

Results: The patients were all female and the age was 54, 62 and 71. The imprint cytology specimens predominant consisted of epithelial cells forming various clusters and foamy macrophages. Epithelial cells had round nuclei, smooth nuclear margin and indistinct nucleoli. Mitotic count including abnormal mitosis absent. On image and gross findings, the tumor showed the well-defined solitary round mass. On frozen section, all lesions were accurately diagnosis as SP. Histologically, the tumor consisted of surface cuboidal and stromal round cells with solid, papillary, sclerosing or hemorrhagic pattern. Immunohistochemical analysis confirmed two-layer epithelial patterns.

Conclusion: The age and sex of patients, well circumscribed image and macroscopical findings and absence of nuclear criteria of malignancy of epithelial cells in cytological materials with infiltration of foamy macrophages are diagnostic clues to diagnosis

accurately. In order to avoid the misdaignosis, cytopathologists need to recognize these findings steadily.

Disclosure of Interest: None declared.

P-104

Rare Two Cases of Respiratory Fungal Infections in Japan

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Objectives: Case 1, We detected *Cryptococcus gattii* in pleural effusion and sputum and bronchial brushing specimen. There are two kinds of Cryptococcosis, C. *neoformans* and C. *gattii*. Usually, *C. gattii* is found in tropical or subtropical area such as in Australia. It is isolated from the trees like eucalyptus, and it causes severer disease compared to C. neoformans. It also affects healthier individuals compared to C. neoformans. Case 2, We detected *Scedosporium* in sputum and bronchial washings from a female with no immunological abnormality. It is reported that *Scedosporium* is found in Tsunami lungs and in opportunistic infections of immunocompromised patients.

Case 1: A 71-years-old man presented to a hospital with common cold-like symptoms, but antibiotics were not effective and he started to have chest pain. Chest computed tomography (CT) scan showed tumorous shadow in his right lower lobe. He also presented bloody sputum and viscous pleural effusion. Cytological examination of the pleural fluid was very characteristic. It showed many organisms with thicker capsules than those of C. neoformans in a bloody background. He was diagnoses as Cryptococcosis with *C. gattii* by a result of fungal culture, and had right lower lobe resection and recovered.

Case 2: A 65-years-old woman was referred to our hospital with fever and productive cough. Chest CT scan showed a cavity with fungus ball in the left lower lobe, and she was suspected to have aspergillosis. However, anti *Aspergillus* antibody was not found in her blood. Cytological examination of her sputum showed lightly brown septate hyphae. The fungus was later identified as *Scedosporium* spp. by a result of fungal culture. She has been treated with VRCZ.

Conclusion: These fungi are rarely seen in Japan, but are emerging in these days. From our experiences we believe that we had better not classify fungi only by cytology. When we found fungus in cytological examinations, we could only characterize whether the fungus is yeast-like or Filamentous fungus and has aseptate or septate hyphae.

Disclosure of Interest: None declared.

P-105

Morphologic Analysis of Cytomegalovirus Infected Cells in Bronchial Washing Cytology: Comparison of Liquid-Based Preparation and Conventional Smear

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Background: Cytopathic effects of cytomegalovirus (CMV) infection have been well described since the first report, however morphologic analysis of CMV infection has not been clearly studied. We examined the difference in detailed cytologic findings in bronchial washing cytology between liquid-based and conventionally prepared smears.

Methods: Bronchial washing cytology was processed by either the conventional preparation (CP) or liquid-based preparation (LBP). Sixty nine cells with typical cytopathic effects of CMV infection were detected on CP slides and 18 cells on LBP slides. Using the image analyzer, area, circumference, major axis and minor axis of the cytoplasm, nucleus and intranuclear inclusion were measured in singly scattered CMV-infected cells and histiocytes as a control.

Results: The mean cytoplasmic area of CMV-infected cells was 1.47 times larger than that of histiocytes in CP and 2.92 times larger in LBP ($p < 0.05$). The mean nuclear area of CMV-infected cells was 2.61 times larger than that of histiocytes in CP and 4.25 times larger in LBP ($p < 0.05$). The nucleus to cytoplasm ratio and intranuclear inclusion to cytoplasm ratio of mean area, circumference, major axis, and minor axis in CP were larger than those in LBP ($p < 0.05$).

Conclusion: The size of cytoplasm, nucleus, and intranuclear inclusion is larger in LBP than in CP in that CMV-infected cells are easily detectable in LBP. However the nucleus to cytoplasm ratio is larger in CP so that differentiation from some atypia of malignancy or regeneration may require caution in CP.

Disclosure of Interest: None declared.

P-106

Usefulness of Morphological Examination of Fungi in Non-Invasive Fungal Rhinosinusitis

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Objectives: Fungal rhinosinusitis can be divided into non-invasive and invasive forms. The non-invasive conditions include localized fungal colonization, fungal ball and allergic fungal rhinosinusitis (AFRS). We performed morphological studies on fungi detected in the paranasal sinus contents of non-invasive fungal rhinosinusitis in immunocompetent patients.

Materials and Methods: The study materials were the paranasal sinus contents obtained from 38 patients diagnosed with non-invasive fungal rhinosinusitis by histological examination. Paraffin-embedded H&E and Grocott-stained sections of the paranasal sinus contents were prepared for histological examination. In addition, squash cytology specimens of unfixed paranasal sinus contents were prepared and stained with Papanicolaou stain. The morphology and background of the fungi were observed.

Results: Thirty-two of the 38 patients were classified into the fungal ball that consisted of a dense accumulation of fungal hyphae. The morphological characteristics of the fungi were consistent with *Aspergillus* species: as a uniform width of hyphae, existence of septa and Y-shaped branching, etc. In 2 patients, a microorganism having fine filamentous elements and assumed to be *Actinomycetes* surrounded the *Aspergillus* fungal ball. Nineteen of 24 samples cultured for fungi were negative. The remaining 6 (of the 38) patients were classified as AFRS. The mucus contained numerous aggregations of eosinophils that showed degenerative changes of the nuclei and cytoplasm, as well as partial degranulation and Charcot-Leyden crystals. The mucus also contained elongated fungal-like hyphae. Filamentous fungi were isolated from 4 of 5 patients for whom culture was performed.

Conclusions: Culture of the paranasal sinus contents in non-invasive fungal rhinosinusitis is often negative. In addition, a long period of time is required to obtain the results. Meanwhile, histological and cytological examinations are helpful in establishing diagnosis. These procedures are relatively simple, and filamentous fungi and the background surrounding the fungi can be observed directly. Furthermore, the fungal species can be inferred. Prompt and accurate diagnosis will facilitate appropriate treatment of fungal rhinosinusitis.

Disclosure of Interest: None declared.

P-107

A Case of Fungus Ball Formed by Aspergilus Niger in Mandibular Sinus with Marked Calcium Oxalate

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Objectives: Although aspergillosis of the respiratory tract can be diagnosed by its culture, the sensitivity of culture was not so high. Recently, it was reported that the presence of calcium oxalate strongly suggested aspergillosis. The most frequent *Aspergillus* sp. is *Aspergillus niger,* and *Aspergillus flavus* follows. A case of aspergillosis of parasinus is herein reported, in which calcium oxalate was markedly found.

Case: A 80-year-old woman came to the hospital with complaint of swelling left cheek. CT showed marked thickening of the mucosa of the left mandibular sinus with calcification, which suggested fungal infection. The lesion was resected radically.

Materials and Methods: The surface of the resected mass was imprinted on slide glasses. The slide glasses were stained with Papanicolaou stain and Grocott stain. Thin sections of the formalin-fixed and paraffin-embedded (FFPE) tissue were stained with H. E., PAS and Grocott. The sections were also used for PCR analysis. Primer sets spanning ITS2 region, which was short enough to enable FFTP DNA to amplify, of Ascomycetes were designed.

Results: Macroscopically, the specimen was composed of black fragile masses. On the cytological specimen filamentous microorganism were scattered individually, and a ball-like cluster of radiating filamentous was found, which was diagnosed actinomycosis. More importantly, elongated hexagonal crystals were seen. Fungal hyphae could not be found at that time, probably due to the blackened background. Microscopic examination for H.E-stained section showed the masses composed of entangled fungal hyphae and sulfur granules of actinomycosis at the periphery. Fungal hyphae had septa and branches ramifying at 45 degrees. Macroconidia of phiale type with vesicles were found, and the color of conidia was black. Grocott staining added to the specimen of imprint cytology showed numerous fungal hyphae with septa. Black ring structures, which suggested vesicles of macroconidia, were found when the specimen of Papanicolaou staining was carefully observed. *Aspergillus niger* was confirmed by homology analysis by BLAST.

Discussion: It is known that fungi produce oxalate and the oxalate reacts to calcium in the human body, which generate calcium oxalate. Therefore, calcium oxalate crystals are generated in the proximity of densely growing fungal hyphae. It was reported that *Aspergillus niger* was the most frequent as calcium oxalate-producing fungi. Fungal hyphae on the slide glass of Papanicolaou staining could not be found since the background was dark due to black-colored conidia of *Aspergillus niger*.

Conclusion: Grocott or PAS should be stained, when calcium oxalate was seen and morphology of macroconidia of Aspergillus niger should be recognized.

Disclosure of Interest: None declared.

P-108

Pulmonary Zygomycosis Caused by Rhizomucor Pusillus Detected in Bronchial Washing Cytology Specimen: A Case Report

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Background: Pulmonary zygomycosis is an acutely fatal infection, which usually occurs in immunocompromised patients including leukemia. Cytology specimen is one of valuable tools for detection of zygomycosis. We experienced a case of pulmonary zygomycosis caused by *Rhizomucor pusillus* infection, of which hyphae was the initial clue to make a correct diagnosis of it.

Case: A 69-year-old man referred to our hospital due to acute lymphoblastic leukemia (ALL). He was treated with first-line induction chemotherapy and followed by consolidation therapy. In transbronchial brush cytology, which was obtained from the tumor mass which showed reversed halo sign appearing in the hilar region of the right lung in CT, a wide, coenocytic, ribbon-like hyphae with wide-angle branching were observed. *Rhizomucor pusillus* was identified by morphology and molecular analysis of culture specimen. He died of ALL, although zygomycosis had been well controlled after right upper lobectomy.

Conclusion: Cytology specimen is a useful tool for detecting zygomycosis to prescribe an appropriate treatment of zygomycosis.

Disclosure of Interest: None declared.

P-109

Cytology Diagnosis of Pneumocystis Pneumonia in Bronchoalveolar Lavage Fluid: Five-Year Experience of NTUH

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Objectives: Pneumocystis pneumonia (PCP) is one of the most common opportunistic infections in immunocompromised patients, particularly those with HIV/AIDS. A definite diagnosis of PCP requires microscopic identification of the cystic or trophic forms of Pneumocystis jirovecii organisms in the respiratory secretion, usually an induced sputum or a bronchoalveolar lavage (BAL) fluid specimen. Selective stains such as Gomori-methenamine silver (GMS), cresyl violet, and toluidine blue O help outline the cell wall of the cystic form of P. jirovecii. Recent development of polymerase chain reaction (PCR) technology has offered another diagnostic option of P. jirovecii, even in the non-invasive oropharyngeal washing specimens, though there is concern about false-positive results. In this study, we aimed to evaluate the frequency of PCP and cytology role in a BAL cytology series of a single tertiary referral center in Taiwan within the five-year period.

Materials and Methods: Clinical and cytopathology features were reviewed from patients receiving BAL examination at the National Taiwan University Hospital between November 2010 to October 2015. PCR assay for P. jirovecii was done if adequate specimens available.

Result: During this study period, there were 388 patients undergoing BAL cytology examinations, among which 31 with a request for P. jirovecii evaluation. A cytological diagnosis of P. jirovecii infection, after identifying foamy alveolar casts with central dot-like structures inside on the smears, had been made in 14 patients (14 males; median 39 years; range 23–58 years) representing 3.6% of all BAL cases. There were 12 patients with HIV/AIDS and two patients with leukemia/lymphoma. Three patients had been confirmed with positive PCR tests, while two patients had been confirmed after Gomori methenamine silver (GMS) staining. Clinical response could be assessed in 12 patients with ten patients to typical anti-PCP regimen trimethoprim/sulfamethoxazole (TMP/SMX), one to dapsone after TMP/SMX allergy, and one to caspofungin after TMP/SMX failure.

Conclusion: Cytology evaluation in BAL fluid is a useful tool for diagnosing PCP in immunocompromised hosts, such as patients with HIV/AIDS and lymphoma/leukemia in this study. Acquaintance with cytology features and ancillary studies will help guide timely patient management.

Disclosure of Interest: None declared.

P-110

Beneficial Effect of Forest Bathing on Elderly Patients with Chronic Obstructive Pulmonary Disease

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Objective: To investigate the effect of forest bathing as a natural therapy for elderly patients with stable chronic obstructive pulmonary disease.

Methods: Eighteen elderly patients with stable chronic obstructive pulmonary disease were randomly divided into two groups. One group was sent to a broad-leaved evergreen forest to experience a 4-day trip, and the other group was sent to a city area in Hangzhou as control. Peripheral blood was collected before and after the experiment, and proportions of NK, NKT-like and $CD8^+$ T cells as well as perforin and granzyme-B expression in these cell populations were analyzed by flow cytometry. Pro-inflammatory cytokines and stress hormones were detected as well. Besides, profile of mood states (POMS) evaluation was used to assess the change of mood state of subjects.

Results: There was no significant difference as for baseline health indicators of the study subjects. No significant change of indicators in the city group was observed after the experiment. While subjects exposed to the forest environment showed a sig-

nificant reduction of intracellular perforin expression. The proportion of CD8 T cells and NKT-like cells expressing perforin decreased significantly after experiment both compared to their baseline (CD8 T cells, 3.02% vs. 22.65%, $P < 0.001$; NKT-like cells, 6.84% vs. 36.22%, $P < 0.001$) and compared to city group (CD8 T cells, 3.02% vs. 10.90%, $P < 0.01$; NKT-like cells, 6.84% vs. 16.87%, $P < 0.05$). Similarly, the proportion of NK cells expressing perforin also decreased compared to baseline (54.32% vs. 93.28%, $P < 0.001$). Levels of pro-inflammatory cytokines and stress hormones also decreased in forest group compared to their baseline (IL-6, 36.92 vs. 97.32 ng/L, $P < 0.01$; IL-8, 88.14 vs. 195.25 ng/L, $P < 0.05$; IFN-γ, 352.74 vs. 845.19 ng/L, $P < 0.01$; epinephrine, 97.63 vs. 197.31 ng/L, $P < 0.05$). Meanwhile, POMS evaluation showed that the scores in the negative subscales were decreased after forest bathing trip.

Conclusion: Taken together, our results indicated that forest bathing has beneficial effect on COPD patients by reducing the inflammation and stress level and thus improve their health condition.

Disclosure of Interest: None declared.

Effusions

P-111

Construction of Cytologic Microarray of Cell Transferred Serous Effusion Specimens and Its Application to Immunocytochemistry

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Objectives: Cell transfer is a valuable technique to transfer cell materials from preexisting cytological preparations to a new slide or slides. This study is to evaluate the utility of cell transfer technique in construction of cytologic smear microarray and its application to immunocytochemical studies.

Materials and Methods: Ninety six cytology smears, including 2 pericardial effusions, 22 ascites and 72 pleural effusions, were transferred to constructed 33-plexed cytological microarray. Each slide was duplicated to perform paired TTF-1 and calretinin immunocytochemical study.

Result: Most of the smeared cells intended to transfer were removed from the original slides with minimal cells loss. The morphological features and immunoreactivity were unaffected by this technique. Comparison of the staining results with available results of immunohistochemical staining, clinical history, and histopathological reports of the same patients reveal that TTF-1 was positive in 32/33 metastatic pulmonary adenocarcinoma, 1/15 in non-pulmonary adenocarcinoma and 0/45 in benign effusions. Immunocytochemical stain of TTF-1 on transferred cytological microarray was sensitive (97%) and specific (96.7%) for detecting metastatic adenocarcinoma of lung.

Conclusion: We therefore conclude that cytology microarray can be constructed by transfer cells from serous effusion cytological smears. The transferred cells on the microarray retained the morphological integrity and immunoreactivity. Simultaneous immunocytochemical studies on multiple cytological specimens can be achieved on a single slide by this technique. Quantity of cells required for microarray construction is minimal. This technique provides opportunity for multiple comparable immunostaining for neoplastic or non-neoplastic diseases of research interest but lacking adequate tissue samples.

Disclosure of Interest: None declared.

P-112

Comparison of Usefulness of Three Epithelial Markers in Effusion Cytology

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Background: Several epithelial markers have been used for immunocytochemistry in effusion cytology. Recently, claudin-4 (CL4) immunocytochemistry was reported to be useful. However, a large-scale comparison of the usefulness of such markers, including CL4, has not been performed.

Methods: In total, 266 cases (169 metastatic carcinomas, 8 malignant mesotheliomas, and 89 reactive mesothelial cells) were selected. Immunocytochemical examinations of cell-block sections were performed: for CL4 and for two representative epithelial markers, Ber-EP4 and MOC-31. We used an arbitrary 4-tiered scale based on both staining intensity and positive-cell percentage among all target cells, and calculated a staining index score (sum of the above two scores).

Results: In a ROC-curve analysis, higher area-under-curve values were found for CL4 than for Ber-EP4 or MOC-31 (0.982, 0.942, and 0.926, respectively).

Conclusions: Although all three markers exhibited good discrimination values in metastatic cancers vs. mesothelial cells in effusion cytology, CL4 could be the best of the three for the above differential diagnosis.

Disclosure of Interest: None declared.

P-113

Immunocytochemical Testing of Exudate from Serous Cavity with Ber-EP4 Epithelial Marker

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Background: In case of adenogenous cancer dissemination on serous membranes Ber-EP4 (Ep-CAM) is the most effective and sensitive marker for differential diagnostics between reactive and metastatic exudate. Its sensitivity is 96%, specificity – 99%. Immunocytochemical (ICC) testing o exudates from serous cavities with BER-EP4 epithelial marker confirms the presence of glandular epithelium in cells exudate. But it can be not only tumorous which be the reason for overdiagnosis of tumor stage dissemination on serous membranes.

Method: Two ICC methods were used – immunoperoxidase and immunofluorescent. Ber-EP4 was used as a marker for epithelial glandular nature. In order to evaluate immunofluorescent reaction a fluorescent microscope Imager1 by 'Karl Zeiss' was used.

Results: Exudates always contain cells of macrophage nature that absorb dye particles which does not mean expression, in this case there is apparent membrane reaction. It must be considered that there is a possibility for false-positive reactions in case of benign ovarian tumor with papillary structures of epithelium on the surface of ovaries, superficial serous papilloma or cystadenofibroma. In our practice there are examples of Ber-EP4 epithelial marker expression in nonneoplastic processes which include endosalpingiosis and endometriosis of abdominal membrane, where intensity of proliferative processes may vary up to atypical. ICC reactions evaluation in exudates testing in case of recurrent surgery must be performed with caution as epithelial cells of the ovary that got to the exudate may store up to 3 months and it is practically impossible to say, whether it is implant or persisting tumor cells. Cuboidal epithelium of lepidic tissue of ovarian cyst that got to ascitic fluid in the course of cyst puncture, as well as ovarian surface epithelium that got to the exudate after ovulation express Ber-EP4 marker.

Conclusion: Ber-EP4 epithelial marker is a reliable marker for diagnosis of adenogenous cancer in serous exudates but rare benign and nonneoplastic processes with cells of glandular epithelium in exudates must be considered.

Disclosure of Interest: None declared.

P-114

How to Make a Definitive Diagnosis of Mesothelioma by Effusion Cytology. Utility of Immunochemical Staining and p16/CDKN2A FISH Using Cell Transfer Method and/or Cell Block Method

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Background: In mesothelioma, body fluid accumulates in early stage (stage I) different from other malignant tumors. Therefore, body fluid cytology is very important for early detection and treatment of mesothelioma. Particularly, the prognosis of pleural mesothelioma in early stage has become better recently because of pleurectomy/decortication. The purpose of this study is to determine whether malignant mesothelioma can be diagnosed definitively by effusion cytology.

Patients and Methods: This study included 13 patients with malignant mesothelioma diagnosed by effusion cytology in the past 10 years in our institute. These patients were considered as having mesothelioma by body fluid cytology from the cytological findings which are characterictic of mesothelioma. Furthermore, immunochemical staining were carried out for a definitive diagnosis using the cell transfer method and/or the cell block method for these patients. Furthermore, p16/CDKN2A FISH was also performed when needed.

Results: 1) Immunochemical staining, Calretinin was positive in13/13 patients (100%), CK5/6 in 13/13 patients (100%), D2-40 in 13/13 patients (100%), WT-1 in 12/13 patients (92%), CEA in 0/13 patients (0%), MOC31 in 0/13 patients (0%), BerER4 in 0/13 patients (0%), p53 protein in 10/13 patients (77%), EMA in13/13 patients (100%), CD146 in11/13 patients (85%), desmin in 0/13 patients (0%). As a result, the 13 patients were definitively diagnosed as having malignant mesothelioma. EMA, CD146 and desmin were especially useful for discrimination of mesothelioma and reactive mesothelial cells. 2) p16/CDKN2A FISH was useful to distinguish mesothelioma cells from reactive mesothelial cells.

Conclusions: Good immunochemical staining results were obtained, enabling the definitive diagnosis of mesothelioma. Immunochemical staining and p16/CDKN2A FISH using the cell transfer method and/or cell block method are important for the definitive diagnosis of mesothelioma by effusion cytology.

Disclosure of Interest: None declared.

P-115

Cytologic Method's Capabilities in Differential Diagnostics of Malignant Mesothelioma

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Diagnostics of malignant mesothelioma and definition of its variants is a highly topical issue according to the data of the International Mesothelioma Interest Group in 2015, consisting of scientists from many countries (Sweden, Australia, USA, Netherlands, Turkey, Japan, France, Italy, Norway, etc.).

This disease is a rare type of malignant tumor and cytological method's capabilities in verification of malignant mesothelioma variants are studied insufficiently in Kazakhstan.

Objective: Explore the cytological method possibilities in diagnostics of malignant mesothelioma variants.

Materials and Methods: We analyzed the material on cytological and histological diagnostics from 4 patient care institutions of Almaty city for the period 2010–2015 from 17 hospitalized patients. Among them – 12 men (70%), mean age – 43; and 5 women, mean age – 55.6. Morbidity rate of malignant mesothelioma per 100,000 of population was 0.7 in Almaty.

Malignant mesothelioma's diagnosis was studied histologically on thoracoscopic biopsy and autopsy material in 6 patients.

Cytological material of pleural and ascitic fluids was analyzed in 11 patients, a liquid-based cytology technique with Papanicolaou stain was used along with routine method.

Results: Analysis of 6 patients with histologically verified diagnosis showed that 1 of them had a clinical diagnosis – exudative pleurisy of unclear etiology; 1 patient had a clinical diagnosis – tuberculosis of peritoneum; diagnosis – incarcerated ventral hernia was in 1 case, 1 patient had a diagnosis – hemothorax with coagulated hematoma and 1 patient had a clinical diagnosis on the autopsy material – lung cancer with metastases into abdominal cavity. Clinical diagnosis – malignant mesothelioma was in 1 case only and was confirmed histologically. Thus, there was a divergence in clinical and histological diagnosis in 5 cases. Malignant mesothelioma was confirmed histologically in all cases of examined patients and epithelial variant was determined in 3 cases, sarcomatoid variant was detected in 1 case and 2 patients had malignant mesothelioma without specifying of variants.

Analysis of 11 patients' data who underwent only cytological examination of pleural and ascitic fluids shows that 4 of them are diagnosed clinically with – cancer metastasis to the pleura, 7 patients had malignant mesothelioma. Later 7 seven of them were diagnosed cytologically with epithelial mesothelioma, 2 patients had sarcomatous variant and 2 had mesothelioma without specifying of variants.

Conclusion: Thus, cytologic verification of malignant mesothelioma is an objective, easily accessible and minimally invasive method, can serve as a diagnostic standard, specifying morphological variants.

Disclosure of Interest: None declared.

P-116

Utility of Liquid-Based Cytology in the Diagnosis of Malignant Pleural Mesothelioma

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Objectives: Malignant pleural mesothelioma (MPM) is a refractory tumor with poor prognosis associated with asbestos exposure. At early stage of MPM, pleural effusions are frequently developed, and the cytological analysis of pleural effusions is effective to detect MPM. It has recently been reported that the cytological diagnosis of MPM supported by ancillary techniques, such as immunocytochemistry and fluorescence in situ hybridization (FISH) analysis, is as reliable as that based on histopathology, and subsequently, guidelines for the cytological diagnosis of MPM have been issued. On the other hand, liquid-based cytology (LBC) was originally developed for analysis of gynecologic cervical smears taking advantages of the high recovery of good preserved cells and the ability to run additional analyses including immunocytochemistry and FISH analysis on the same sample. LBC has also been progressively used for preparing fine needle aspiration specimens. However, the utility of LBC is unclear in the diagnosis of MPM using pleural effusions. The purpose of this study is to establish morphological features of MPM cells in LBC and to determine the utility of LBC for diagnosis of MPM.

Methods: Specimens of MPM cell lines and MPM pleural effusions were prepared using conventional smear and Cellprep LBC. Cell blocks were also prepared from MPM cell lines and MPM pleural effusions. Specimens were stained with Papanicolaou, immunostained with BRCA1-associated protein 1 (BAP1) antibody, and used for FISH analysis of *CDKN2A* gene.

Results: Morphological features in the LBC specimens were similar to those in conventional smear; characteristic features of MPM cells, such as cell-in-cell engulfment with or without hump formation, multinucleate cells, and larger berry-like cell aggregates, were observed in LBC specimens. In addition, LBC specimens were suitable for BAP1 immunostaining and *CDKN2A* FISH analysis, which were important to distinguish MPM from reactive mesothelial proliferations.

Conclusion: These results indicate that LBC of pleural effusions is highly useful in diagnosing MPM.

Disclosure of Interest: None declared.

P-117

Characteristics of Serous Effusions: Review of Consecutive Cases Diagnosed in Two Years in a Single Institution

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Objective: The aim of this study is to review the serous effusions examined in our institution, to investigate age and sex distribution, malignancy rates, most common primary sites, and to look for the relation between the amount of fluid and malignancy.

Materials and Methods: The records of 1204 consecutive serous effusions from 946 patients diagnosed between 2013–2014 were retrieved from the archives. The series was composed of 881 pleural, 298 peritoneal and 25 pericardial effusions from 662, 261 and 23 patients, respectively. Cytological diagnosis, results of immunohistochemistry and all available clinical data were correlated.

Results: Male to female ratio was 1.2/1 with a mean age of 59.1 and 58.6 respectively. 60% of pleural, 44% of peritoneal effusions were from males. Although statistically insignificant, malignancy rates were higher in females for both cavities. 26% of all specimens were diagnosed as malignant, 8.4% as atypical, and 69.9% as benign. The malignancy rate was significantly higher in peritoneum than pleura ($p < 0.001$). Patient based malignancy rates were 25.2%, 38%, 30.5% for pleura, peritoneum and pericardium, respectively. Most common primary sites for pleura were lung (34%), hematolymphoid (14%), breast (11%); for peritoneum gastrointestinal/pancreatic (35%), gynecologic (30%), hematolymphoid (6%); and for pericardium lung (43%). 25% of lung carcinomas detected in pleura, and 31.4% of gastrointestinal/pancreatic carcinomas diagnosed in peritoneum were in females. 19 patients with pleural, 15 patients with peritoneal, and 4 patients with pericardial effusions were under age 18; and the malignancy rate was highest in pleura among these cases (21%), lymphoma being the most common primary. Immunohistochemical studies were performed overall in 23.4% of the cases. 150 patients with pleural, 29 patients with peritoneal effusions had more than one sample; repeat taps in 9 patients with pleural effusions resulted in major diagnostic change (from benign to malignant). Specimens from peritoneal cavity were higher in amount in comparison to pleural cavity with medians 250 ml and 30 ml, respectively; when the medians are considered as cut-off levels, a significant difference was detected in malignancy rates between the volumes under and over these values ($p < 0.05$). Malignancy rate increased constantly as the amount of fluid increased.

Conclusions: The highest malignancy rate was in peritoneal effusions. Lung and breast for the pleural cavity, gastrointestinal/pancreatic and gynecologic for the peritoneal cavity, hematolymphoid for both cavities were the most common primary sites. Repeat taps resulted in major diagnostic change in a small percentage of patients. Malignancy rate increased with the amount of fluid examined.

Disclosure of Interest: None declared.

P-118

Comparative Study on the Method of Lysis Solution for Eliminating Red Blood Cell from Bloody Fluid

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It is very important in cytology preparation in bloody fluids to make a clean background of cytospin in good quality for correct diagnosis. This is a big problem for cytocreener and pathologists who screen abnormal cells. In general, bloody fluid is not compatible with routine cytospin due to a lot of blood in the background. This research focused on inventing the lysis solution that can eliminated red blood cells from the bloody fluid specimens while preserve cell's morphology and antigenicity. This will allow cytospin preparation hence improve diagnosis accuracy and enable further investigation.

Objective: To compare 4 lysis solutions No.1, No.2, No.3 and No.4, which one can make clean background and well preserved cytomorphology.

Materials and Methods: Cytospin preparations were made by 4 types of lysis solution. In this research, we studied4 solution which were.

No.1: Carnoy's fixative, No.2: 1% glacial acetic acid in 95% ethyl alcohol. No.3: Lysis solution A. (Cytolyt) and No.4: Lysis solution B.(CytoRich Red) Random 30 bloody fluid specimens from pleural and abdominal cavity were treated with all 4 solutions mentioned above. Bloody fluid 50 ml was centrifugetion at 2,000 rpm for 10 minutes. Supernatant was removed. Add 0.2 ml of sediment into 10 ml of NSS mixed and use 3 ml in chamber for cytospin. Set the speed 1250 rpm/5 minutes. Finished centrifuge fixed slide one in Carnoy's fixative and other in 1% glacial acetic acid in 95% ethyl alcohol for 5 minutes then removed slides to fix in 95% alcohol. Add 0.2 ml of sediment in 10 ml solution A and B mixed on vortexor and allow to stand in solution A and B for 30 minutes. The specimens are processed by cytospin preparation speed 1250 rpm/5 minutes and staining with Papnicolaou stain. The lysis formulas were evaluated by the success rate of clear and clean background in cytospin preparation. Slides from successful clearing were blind examined by one pathologist and two cytoscreeners. Graed the quality of the cytospin slides by 4 categories: the background, the preservation of cell morphology, the quality of dye stain, and overall satisfaction.

Conclusion: Success rate of eliminate RBC in background 1% glacial acetic acid 25%, Carnoy's fixative 60%, lysis solution A.(Cytolyt) 84% and lysis solution B.(CytoRich Red), 86% had higher score in pathologist' satisfaction. Although, Carnoy's fixative and 1% glacial acetic acid in 95% ethyl alcohol are cheaper than two solution A. and B. but had unpleasant and irritant smell, so we will introduce solution A and B lysis solution as a in laboratory's routine bloody body fluid preparation. This study are can modified and reduced the volume of solution A and B from 30 ml.(as recommended protocol.) which can simplified reduce processing time and cost.

Disclosure of Interest: None declared.

P-119

The Utility of Nucleolar Size as a Reliable Cytomorphological Parameter in the Assessment of Malignant Cells in Pleural and Peritoneal Effusions

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Introduction: Cytological interpretation of metastatic malignancy in pleural and peritoneal effusions requires the application of several cytomorphological parameters. Recognition of the variation in cytomorphological appearance of benign mesothelial cells in the setting of reactive changes is important to avoid the erroneous interpretation of metastatic malignancy. Thus, a nuanced understanding of certain key cytomorphological features is important in the area of effusion cytology.

Aim: In this study, nucleolar size of benign versus malignant cells in pleural and peritoneal cytology was compared to assess its reliability as a morphological indicator of malignancy.

Methods: From 2014, up to 100 cases slide of effusions cytologies were reviewed. The nucleolar sizes of individual cells were compared between cytologically benign and malignant cells within the processed cytological material. Nuclear parameters were calculated using 3DHISTECH CaseViewer imaging software.

Results: In the hundred cases reviewed, a noticeable and significant increase in nucleolar size in comparison to the reactive mesothelial cells component was observed. The mesothelial cells exhibit smaller, more peripherally located nucleoli, whereas the cytological malignant cells possessed larger centrally located nucleoli. Significant differences in nucleolar sizes were observed between benign and malignant cells in the respective cases.

Conclusion: In summary, nucleolar size can act as a reliable cytomorphological indicator of malignancy. This cytomorphological feature is vital parameter in the assessment of peritoneal and pleural fluid cytology. These are findings from a pilot study which we will expand further upon the strength of our current findings.

Disclosure of Interest: None declared.

P-120

A Case of Malignant Lymphoma Initially Diagnosed as Primary Peritoneal Carcinoma

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Introduction: Intra-abdominal cancer often present with severe carcinomatous pleurisy and clinicians sometimes consider neoadjuvant or empiric chemotherapy based on clinical and/or cytological findings. However, it is challenging to predict the histological type only by cytology. We report a case of malignant lymphoma that was initially diagnosed and treated as peritoneal carcinoma.

Case: A 66-year-old woman presented with shortness of breath on exertion for two months. A plain X-ray revealed pleural fluid. Adenocarcinoma was suggested[m1] by initial pleural fluid cytology. Computed tomography showed 5-cm-sized multiple intra-abdominal masses with multiple retroperitoneal lymph-adenopathy, which suggested peritoneal dissemination. Although the uterus and bilateral ovaries were normal looking except for leiomyomas, serum CA125 was elevated (1064 U/ml). She was then referred to our hospital and diagnosed with peritoneal carcinoma based on clinical and cytological data. She received a cycle of dose-dense TC (paclitaxel plus carboplatin) as neoadjuvant chemotherapy, but did not respond to the therapy. Therefore, pleurodesis and cytoreductive surgery were performed. The markedly enlarged mesenteric lymph nodes constricted the jejunum. Retroperitoneal lymphadenopathy was observed, but there were no peritoneal dissemination. The enlarged mesenteric lymph nodes were resected and carcinoma was denied by frozen section. Subsequently, total hysterectomy, bilateral salpingo-oophorectomy, segmental resection of the jejunum (+D1 dissection) and segmental omentectomy were performed.

Cytological Findings of Pleural Effusion and Ascites: In pleural fluid cytology, the majority of obtained cells was thought to be reactive mesothelial cells; however, a few atypical cells with enlarged nuclei and increased chromatin were detected. In ascites cytology, reactive mesothelial cells and normal-looking lymphocytes were detected.

Histopathological Findings: Uniform small lymphocytes with a lymphoid follicle-like structure were observed in the mesenteric lymph nodes. These cells were positively immunostained for CD20 and bcl-2. Pathological diagnosis was follicular lymphoma. The peritoneum focally demonstrated papillary proliferation, indicating ascites-associated reaction. No malignancy was observed in the uterus and ovaries.

Conclusion: In cases of unknown primary malignancy with pleural effusion, pleural fluid cytology is expected to provide diagnostic clue; however, differential diagnosis is not always easy. In the present case, the patient was strongly suspected of carcinomatous pleurisy from clinical data, which prevented us from excluding the possibility of adenocarcinoma from the cytology of a few atypical cells.

Disclosure of Interest: None declared.

P-121

Cytology of Human Herpes Virus 8-Unrelated Primary Effusion Lymphoma-Like Lymphoma

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Objectives: To describe the cytomorphologic, immunocytochemical and molecular features of one case of human herpes virus 8 (HHV8)-unrelated primary effusion lymphoma (PEL)-like lymphoma.

Materials and Methods: A 73-year-old male was studied because of dyspnea and recurrent pleural effusion during the last 18

months. Three years before he was diagnosed of systemic leishmaniasis with reactive myelopathy in a bone marrow biopsy. Imaging techniques and physical examination confirmed a bilateral pleural effusion and excluded lymphadenopathies or any other organ enlargement. The pleural fluid was evacuated and processed for cytological, immunocytochemical and molecular study and a pleural biopsy was performed.

Result: Cytological examination of the pleural fluid showed a highly-cellular sample composed of medium-to-large, epithelioid neoplastic cells with polygonal cytoplasms and eccentrically located, enlarged, irregularly contoured nuclei with coarse chromatin and one or more prominent nucleoli. Bi or multinucleated atypical forms were observed. Mitotic figures were easily seen. The neoplastic cells appeared predominantly singly or in scarce non-cohesive, small groups, together with a discrete number of small lymphocytes, some neutrophils and occasional mature plasma cells. Eosinophils were not significantly increased. The neoplastic cells were negative for calretinin, cytokeratins, synaptophysin, HMB45, OCT3/4, CD68 and vimentin and showed intense and diffuse positivity for CD45. A preliminary diagnosis of 'lymphoma of anaplastic pattern' was rendered. An expanded immunocytochemical panel revealed the following profile: CD20 +, CD10-, CD138-, bcl-2 +, MUM-1 +, with isolated cells positive for bcl-6 and CD30 and a proliferative index (ki67) of 60%. Neoplastic cells were negative for HHV8 and positive for Epstein-Barr virus. Molecular studies demonstrated a clonal proliferation with c-myc amplification (8q24 region). The pleural biopsy resulted nondiagnostic.

Conclusion: Similarly as other cases of this recently described entity, our report is of a primary large B-cell lymphoma of the serous cavity in an elderly, immunocompetent patient with a history of chronic inflammation. The negativity for HHV8 and c-myc/8q24 status separates this entity from HHV8-related PEL. More clinicopathological descriptions of this rare entity, still not included in the WHO classification and that seems to have a better prognosis that its HHV8-related counterpart, are needed in order to define its characteristics. Due to its manifestation in serous fluids, without involvement of other tissues or organs, the diagnosis rests mainly in cytology.

Disclosure of Interest: None declared.

P-122

Burkitt Lymphoma Found in a Pregnant Woman and Diagnosed by Ascitic Cytology: A Case Report

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Introduction: Burkitt lymphoma (BL) is one of the highly aggressive, diffuse non-Hodgkin B-cell lymphomas with abnormal karyotype of t(8;14)(q24;q32) and *MYC* gene rearrangement. On the other hand, coexistance of malignancy and pregnancy in one patient is very rare. In this time, we report a case of BL found in a pregnant woman.

Case: An about 30-year-old woman, who was 3 months pregnant, was evaluated for right lower abdominal pain and palpable mass. Abdominal computed tomography (CT) scan demonstrated massive ascites and an intraabdominal huge masses located in the ileocecal region, ascending colon and pancreas. Ascites material played an important role in definitive diagnosis of this case because histological examination could not be done on account of her worse condition. On the next day after admission to our hospital, the patient was diagnosed as BL and started to be treated with chemotherapy. Eleven months later, CT scan could not demonstrate overt ascites and mass.

Morphology and Other Examinations: Ascitic cytology revealed malignant lymphoma cells, characterized by a uniform population of non-cohesive lymphoid cells with non-cleaved nuclei, prominent nucleoli, and scanty basophilic cytoplasm. Multiple cytoplasmic and nuclear vacuoles were also detected. Flow cytometry showed that lymphoid cells were largely positive for CD10, CD19, CD20 and lambda chain and negative for CD2, CD3, CD5 and kappa chain. Immunostaining showed most of lymphoma cells were positive for CD20, Bcl-2 and Ki-67. Moreover, split signals were detected in many lymphoma cells by fluorescence *in situ* hybridization with *MYC* flanking probes of 8q24 region (89.5%). These findings indicated BL.

Conclusions: We experienced a rare case demonstrating the importance of not only recognizing the cytologic features of BL but also making the best use of ascitic cells for rapid diagnosis and treatment.

Disclosure of Interest: None declared.

P-123

Plasmacytoid Urothelial Carcinoma with Malignant Ascites Morphologically Mimicking Lymphoma: A Rare Case Report

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Objectives: The aim of this case was to report a rare malignant ascites origin from plasmacytoid urothelial carcinoma (PUC) which morphologically mimicks lymphoma and the diagnostic value of uroplakin.

Materials and Methods: Ascites was centrifuged at 1200 rpm for 5 min, and the supernatant was discarded. The resulting sediments were used to make two cytospine slides. Both of them was put immediately in 95% alcohol for PAP stain. For immunocytochemical stains, a repeat fresh sample was obtained and cytospin slides were made from the centrifuge deposit. 10% formalin-fixed biopsy specimens from urinary bladder tumor and colonic tumor were processed for H&E staining and later for immunohistochemistry.

Results: A 73-year-old male present with tarry stool, bilateral lower extremities and scortum edema associated with abdominal

distension for two weeks. The PAP smear of ascites demonstrated medium sized discohesive tumor cells with eccentric nuclei, irregular nuclear membrane and scant vacuolation. The immunocytochemical stains of tumor cells include CK (+), CK7 (+), uroplakin (+), CEA (–), B72.3 (–), CK20 (–), CDX2 (–), CD3 (–), CD20 (–) and calretinin (–). Metastatic poorly differentiated urothelial carcinoma was first considered. Cystoscopic biopsy demonstrated PUC. Endoscopic colonscopic biopsy showed primary moderately differentiated colonic adenocarcinoma. Abdominal CT reveals diffuse urinary bladder wall thickening and multiple enlarged para-aortic lymph nodes as well as massive ascites and carcinomatosis. The patient died one month latter after 1st course chemotherapy.

Conclusion: PUC is rare and aggressive variant of urothelial carcinoma (UC). Malignant ascites caused by UC is uncommon. Cytological features consist of (1) discohesive single cells, (2) eccentric nuclei, (3) moderately nuclear variation, (4) coarse nuclear chromatin, (5) small or no nucleoli, and (6) irregular nuclear membrane resembling plasmacytoma, lymphoma and lobular carcinoma. The patient has synchronous double primary cancer including colonic adenocarcinoma and PUC. Uroplakin is sensitive (40–80%) and specific marker for urothelial cells and may be helpful in cytological diagnosis and AJCC staging.

Disclosure of Interest: None declared.

P-124

An Adult Granulosa Cell Tumor of Ovary Metastasized to Pleural Effusion: A Case Report and Literature Review

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Objectives: Adult granulosa cell tumors (AGCT) are low-grade neoplasms. Morphologically they may exhibit a variety of histologic patterns, but cytology is usually bland with oval nuclei, longitudinal nuclear grooves and a low mitotic rate, exhibiting minimal atypia. They are usually confined to the ovary, but metastasis may occur indicating poor prognosis. Here we introduce an AGCT metastasized to pleural effusion, which is rare and should be differentiated from low-grade adenocarcinoma and malignant mesothelioma (MM).

Materials and Methods: A 42-year-old lady with lower right abdominal pain was admitted to our hospital. A CT scan indicated multiple nodules on the surface of liver and peritonea with circumscribed ascites. Multiple nodules were also observed in two upper lungs with right pleural fluid. Previously she had right adjunct resection of ovary AGCT 5 years ago. And she has been under regular follow up ever since. Direct smear and cell block were made from the pleural effusion. There were many three-dimensional cell aggregates with small oval nuclei. Nuclei were less chromatic with a low N/C ratio. Small nucleoli and occasional nuclear grooves can be observed. It stained strongly positive for mesothelial markers of CR, WT1, D2-40 and Desmin, negative for EMA, CEA and TTF-1. A weak stain of a-inhibin was observed in our case to confirm the final diagnosis of AGCT metastasized to pleural fluid.

Results and Conclusion: As AGCT in effusion looks bland and stains positive for mesothelial markers of WT1, CR, D2-40. Differential diagnosis should exclude reactive mesothelial hyperplasia, MM and low grade adenocarcinoma. Immunopositivity of both mesothelial markers and desmin exclude MM. Immunonagativity of CEA, EMA and TTF-1 declines the diagnosis of adenocarcinoma. a-inhibin plays an important role in confirming the AGCT metastasized to the pleural effusion.

Disclosure of Interest: None declared.

P-125

Pericardial Effusion Neoplastic Metastasis to Lung Adenocarcinoma. Description of a Case

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Introduction: Payments neoplastic of serous cavities are usually bleeding and reform over a short time since drainage. They are characterized by the presence of many tumor cells that have either individually or in three-dimensional aggregates. Payments can be divided into primary (mesothelioma) and secondary (metastatic).

Case Report: A man of 65 years, admitted to emergency at the U.O. Internal Medicine Hospital of Crotone diagnosed with massive effusion and subsequent cardiac tamponade. Where ultrasound guided pericardiocentesis, whereby some are drained (800 cc) of fluid and blood serum, and that is made cytology test that the microbial test.

Material and Method: For cytology test were used 40 ml of payment. From the morphological point of view, on a background blood, it highlights many aspects of tubular aggregates sometimes branched neoplastic elements consisting of medium and large size with rounded nuclei, chromatin fienement granular cytoplasm and more or less abundant. Immunocytochemical investigation is performed by testing the following antibody panel: Cytokeratin AE1/AE3, CK7, TTF1, CD56, P63, Chromogranin.

Results and Conclusion: The neoplastic cells showed positive for TTF1, Cytokeratin AE1/AE3 and CK7, but were negative for DC56, P63 and Chromogranin. Thus was diagnosed localization, metastatic pericardial of non – small cell lung cancer whose morphologic and immunophenotypic profile is consistent with adenocarcinoma. Carcinoma of the lung, breast, lymphomas and leukemias are the most common causes of malignant pericardial effusion. The diagnosis of malignant pericardial effusion can be extremely difficult because the clinical manifestations are insidious and may mimic more common diseases.

Disclosure of Interest: None declared.

P-126

Diagnostic Difficulties and Pitfalls of Soft Tissue Sarcoma in Effusion Cytology

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Objectives: Soft tissue sarcomas are relatively rare compared to other malignancies. Differentiating sarcomatous cells from mesothelial cells and metastatic carcinomatous cells in effusion cytology is often difficult due to the limited experience and lack of specific feature. We retrospectively reviewed effusion cytological findings of sarcoma diagnosed in our hospital.

Materials and Methods: Of the 1721 effusion specimens sent for cytological examination and cell block preparation from 2005–2012, 9 (0.5%) were soft tissue sarcomas. All cases were prepared for conventional smears stained with Liu's and Papanicolaou stains. Cell blocks were prepared for H&E stain and immunostains.

Results: The cases included rhabdomyosarcoma (n = 5 in 3 patients), epithelioid hemangioendothelioma (EHE) (n = 1), desmoplastic small round cell tumor (DSRCT) (n = 1), pleomorphic sarcoma (n = 1), and angiosarcoma (n = 1). All rhabdomyosarcoma cases occurred in children, and showed discohesive small to medium sized blue round cells with scant cytoplasm, nuclear molding, round, or irregular hyperchromatic nuclei. The ascitic fluid specimen of DSRCT occurred in a young male adult. The tumor cells formed papillary or spherical nests with smooth contour and pseudolumina, mimicking metastatic adenocarcinoma. The EHE case showed scattered individual or small clusters of atypical epithelioid cells with enlarged nuclei, irregular nuclear contour, prominent nucleoli, and vacuolated cytoplasm. Occasional cytoplasmic lacunae were seen. The cases of pleomorphic sarcoma and angiosarcoma had only scanty atypical cells in the effusion specimen, precluding definite diagnosis in effusion cytology. Immunostaining was helpful in diagnosis of 7 specimens, except the cases of angiosarcoma and pleomorphic sarcoma due to scanty atypical cells, no known previous history of malignancy, and no suitable panel of immunostain for pleomorphic sarcoma.

Conclusion: Soft tissue sarcoma shows a variety of morphology in effusion specimen and may mimic mesothelial cells and metastatic carcinoma. Awareness of the age of the patient and previous malignancy are mandatory. Careful preparation skills can increase the yield of viable tumor cells, making it more suitable for ancillary studies. Application of the appropriate panel of immunostain helps for proper interpretation of effusion cytology.

Disclosure of Interest: None declared.

Urine

P-127

Paris Interobserver Reproducibility Study

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Objectives: In concert with the 2015 Publication of the Paris System for Urinary Cytopathology, a web-based inter-observer study, sponsored by the American Society of Cytopathology (ASC) and International Academy of Cytology (IAC), was performed to evaluate volunteer participant diagnostic concordance with the 'expert panel' diagnostic interpretations.

Material and Methods: Participants of various levels of training and certification were recruited through national and international cytopathology professional societies. The survey was constructed using the Qualtrics® (Provo, Utah, USA) survey and analysis software package made available through the Division of Information Technology at the University of Wisconsin. Study participants evaluated 85 previously unpublished web images chosen from the Paris System (TPS) Atlas image set, prior to the release of the TPS Atlas. These images spanned diagnostic categories and included typical and indeterminate cytomorphology. Demographic information was collected on the level of training, practice patterns, and experience. Participation was restricted to those correctly answering two basic cytopathology questions.

Results: 1,340 persons attempted access to the website, and 698 correctly answered the qualifying questions. Geographically, 40% of respondents came from outside the US. Distribution of certifications were: 28% cytotechnologists, 6% specialist cytotechnologists, 10% pathologists with AP certification, 38% pathologists with cytopathology certification, 4% fellows and 10% other. Distribution of practice types was: 38% academic institutions, 21% private hospitals and 17% private commercial/laboratories. There was similar diagnostic agreement across certifications including: cytotechnologists, specialist cytotechnologists, anatomic pathology certified pathologists, cytopathology subspecialty certified cytopathologists and participants identifying themselves as International Academy of Cytology certified. However, as expected, board certified cytopathologists and specialist cytotechnologists had the best performance. Practice type (academics vs. non-academic), and country (US vs. international) did not prove to be major factors in concordance. The best agreement was found for Negative for High Grade Urothelial Carcinoma (NHGUC)(71%), Low Grade Urothelial Neoplasm (LGUN)(62%) and High Grade Urothelial Carcinoma (HGUC) (57%). Agreement for Atypical Urothelial Cells (AUC)(37%), Suspicious for High Grade Urothelial Carcinoma (SHGUC)(36%) and Other Malignancy (23%) categories showed lower concordance.

Conclusions: The most important factor in image reproducibility was the *a priori* classification of image difficulty rather than participant training or experience. Specifically, agreement was higher for Paris categories of NHGUC and HGUC than for other categories.

Disclosure of Interest: None declared.

P-128

The Application of the Paris System on Urine Cytology: Beijing Hospital Experience

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Objectives: To evaluate the utility of the upcoming classification scheme (The Paris System) of urinary cytology in detection of high-grade urothelial carcinoma (HGUC) and low-grade urothelial carcinoma (LGUC).

Materisals and Methods: A computerized search of our laboratory information system was performed for all urine cytology cases from 2012 to 2015 processed by the ThinPrep™. We included only cases with correlating surgical pathology within 6 months after the urinary samples were obtained. The original cytological diagnoses were reclassified according to TPS, and these cytological diagnoses were then correlated with the follow-up surgical pathology diagnoses.

Results: A total of 571 urine samples with histopathologic follow-up were identified. The reclassified cytologic diagnoses included negative for malignancy (NM) 137; atypical urothelial cells of Uncertain Significance (AUC-US) 89; atypical urothelial cells suspicious for high grade urothelial carcinoma (AUC-H) 103; low grade urothelial neoplasm (LGUN) 76; high grade urothelial carcinoma (HGUC) 166. More than one-half of patients (63%) who had biopsy-confirmed high-grade urothelial lesions had a proceeding cytologic diagnosis of AUC-H or HGUC. AUC-H and HGUC are associated with high-grade urothelial lesions in 84% and 91% of the cases and show statistical significance when compared with AUC-US or NM ($P < 0.05$). Only 23 percent of patients who had biopsy-confirmed low-grade urothelial carcinoma had a proceeding cytologic diagnosis of LGUC.

Conclusion: The TPS is useful and effective in identifying patients with high-grade urothelial lesions, but has a fair accuracy for the diagnosis of low-grade urothelial lesions.

Disclosure of Interest: None declared.

P-129

A New Simple and Comprehensive Reporting System of Urinary Cytology in Japan

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Objectives: Since Papanicolaou's first publication in 1947 on urinary cytology classification, Papanicolaou's 5 stratified classification has long been accepted as the standard reporting system of urinary cytology in Japan up to the present. During this period, some problems have been taking place in this field, such as some local rules developing, vague distinction of each stratified categories, disunion of classification and most doctors looking the Papanicolaou Class number only. On the other hand, some new proposals on urinary cytology have been appeared on the journals in the English-speaking countries. On that background, Japanese Society of Clinical Cytology launched a committee for creating a new reporting system harmonized with other foreign systems and unifying many domestic systems in November, 2012. Here, we present a newly made the Reporting System of Urinary Cytology in Japan.

Materials and Methods: The board of the JSCC founded a committee for unify and create a reporting system of urinary cytology on the November, 2012. The committee consisted of 38 members including urologists, pathologists, cytopathologists and cytotechnologists. The committee collected local systems used in Japan and literatures concerning the reporting systems for urinary cytology, and discussed deeply on (1) urinary cytology being a diagnosis or screening, (2) how different from pathological diagnosis system, (3) best way to unify the local rule into the standard system in Japan, (4) up-grading an inter-observer agreement and (5) how a new Japan system harmonize to the Paris System.

Results and Conclusions: The committee got following results in order to unify the reporting system of urinary cytology in Japan, and harmonize it with the Paris System. (1) Urinary cytology is a risk evaluation system, but a few exceptions, because of exfoliative cytology, (2) urinary cytology categorization should be simple, although some comments is necessary, and (3) a urinary cytology is a risk stratified classification. From those consensuses in the committee, a newly created reporting system was build and consented by the board of JSCC. This was a following. (1.1) statement of adequacy, (1.2) Negative, (1.3) atypical cells, (1.4) suspicious for malignancy, (1.5) malignant and (2) comments.

Disclosure of Interest: None declared.

P-130

Application of the 2015 Japan Reporting System to Urinary Cytology

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Objectives: As the reports of urine cytology in Japan have been based on Papanicolaou classification, sometimes being modified in each local areas, several confusions have been brought about due to the inconsistency in the classification of urine cytology. Recently in Japan the new report style of the classification of urine cytology has been established. The 2015 Japan reporting system for urinary cytology has classified diagnosis into four categories (negative, atypical cells, suspicious of malignancy, malignant) and histological types must be estimated. We have been using modified Papanicolaou classification composing of seven categories (Class I, II, IIIa, III, IIIb, IV, and V). This time we evaluated the 2015 Japan reporting system for urinary cytology retrospectively comparing with traditional reporting system based on Papanicolaou classification and examined sensitivity of urothelial carcinoma.

Materials and Methods: First we examined the sample distribution in Papanicolaou classification and the incidence of cancer using 2535 cases of urine samples from January 2013 to September 2014. Then we applied to adjust these data to the 2015 Japan re-

porting system. Finally we examined the sample distribution in the 2015 Japan reporting system and the incidence of cancer using 1928 cases of urine samples from October 2014 to September 2015.

Results: In the urine samples from January 2013 to September 2014 resulted in as followed, Class I, II, IIIa were negative, Class III were atypical, Class IV were malignant suspicions, Class V were malignancies. In the urine samples from October 2014 to September 2015 resulted in as followed, inadequate were 3 cases (0.3%), negative were 1651 cases (86%), atypical were 124 cases (6%), malignant suspicions were 62 cases (3%), malignancies were 86 cases (4%). The incidence of cancer results in as followed, negative were 5%, atypical were 41%, malignant suspicions were 82%, malignancies were 100%. The sensitivity to identify high-grade urothelial carcinoma remains 96%. The sensitivity to identify low-grade urothelial carcinoma remains 67%.

Conclusion: The results of the 2015 Japan reporting system for urinary cytology in our institution were almost favorable. But the estimation of low-grade urothelial carcinoma was seemed to be difficult in voided urine.

Disclosure of Interest: None declared.

P-131

A Case Report with Cytological and Immunocytochemical Features of Renal Cell Carcinoma Associated with Xp11.2 Translocation/TFE3 Gene Fusion

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Background: Xp11.2 translocation renal cell carcinoma (RCC) is a rare pediatric neoplasm harboring gene fusions involving TFE3, which plays an important role in cell proliferation and survival. We herein present cytological and histopathological features of RCC associated with Xp11.2 translocation/*TFE3* gene fusion.

Case Report: A 14-year-old Japanese boy presented at our hospital with complaints of acute abdominal pain (right side), macrohematuria, fever and 10 kg body weight loss. Abdominal CT, MRI and PET-CT scan showed a large mass lesion with calcification in the right kidney, compressing the vena cava inferior and metastasis to the renal hilar lymph nodes. Right radical nephrectomy was performed according to the clinical diagnosis of RCC or Wilms' tumor. A frozen section diagnosis during the operation reported papillary RCC. However, cytology of imprint touch smears from the cut-surface of the tumor led us to suspect Xp11.2 translocation RCC, because epithelial cancer cells showed a positive reaction for TFE3 in the nuclei of neoplastic cells proliferated with a papillary pattern. Histopathology revealed a biphasic population of neoplastic cells, large epithelioid cells with voluminous eosinophilic cytoplasm and severe nuclear atypia and smaller cells with clear cytoplasm and small round nuclei. There were a few psammoma bodies. Immunohistochemistry showed strongly positive nuclear staining of TFE3 protein in the cancer cells. A RT-PCR analysis of an unfixed and fresh tumor sample showed SFPQ/PSF-TFE3 (+).

Conclusion: Knowledge of distinctive morphological and immunostaining features of this tumor can help to accurately diagnose this rare subset of translocation associated RCC in routine pathological diagnostic procedures.

Disclosure of Interest: None declared.

P-132

Inter-Observer Variability in the Interpretation of an Immunocytochemical Stain for Telomerase in Urinary Tract Cytopathology Specimens

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Objectives: Screening for high grade urothelial carcinoma (HGUC) by urinary tract (UT) cytology is limited by its relatively poor sensitivity. Although several ancillary tests have been developed to address the clinical need for improved UT diagnostic utility, none have gained widespread acceptance. Telomerase is an enzyme that is highly expressed in many urothelial carcinomas and immortalizes tumor cells by maintaining telomere length as the cells divide. Recently, we demonstrated an immunocytochemical method to detect telomerase in UT specimens. While this technique showed promise, the results relied on the observations of only two pathologists. Furthermore, positive cases were found to be associated with a high background signal. While this limited definitive interpretation in these cases, it was believed to be secondary to the presence of telomerase in the supernatant. In the current study, we examined whether the stain, including the background artifact, could be reliably interpreted among a larger number of observers.

Materials and Methods: Ten freshly voided urine specimens were stained in the immunopathology laboratory along with control slides using a telomerase-specific antibody (ab) (Anti-hTERT ab, SCD-A7) developed and provided by Sienna Cancer Diagnostics Ltd. The slides were reviewed blindly by five cytopathologists and one cytotechnologist. Each reviewer interpreted the stain as positive, negative, or equivocal and assessed the background staining pattern as dirty or clean. Fleiss' kappa coefficient was used to measure inter-rater reliability.

Results: The mean age was 66.4 years and 60% were male. The interpretation of the ten cases among the six reviewers had a sensitivity of 67.6% and specificity of 78.3%. There was 100%, 60%, and 0% agreement in the interpretation of seven, two, and one cases, respectively. There was 100%, 66.7%, and 0% agreement in the assessment of the background staining pattern in seven, two, and one cases, respectively. Fleiss' kappa for the interpretation of the stain was 0.251 (95% CI [0.13, 0.37], $p < 0.001$), indicating fair agreement among reviewers. Fleiss' kappa for the assessment of the background was 0.744 (95% CI [0.58, 0.90], $p < 0.001$), indicating substantial agreement among reviewers.

Conclusion: A telomerase-specific antibody test for UT specimens shows fair agreement among reviewers when interpreting the stain and substantial agreement in assessing the background artifact. The background artifact, which has a strong association

with HGUC on subsequent biopsy, had excellent inter-observer concordance. This data suggests that the test can be easily interpreted among practicing pathologists.

Disclosure of Interest: None declared.

P-133

Expression of Vimentin in the Differentiation between Reactive Urothelial Cells and Urothelial Carcinoma Cells in Voided Urine

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Objective: Reactive urothelial cells (RUCs) in calculi show features of an atypical repair reaction. Therefore, differentiation between RUCs and urothelial carcinoma cells (UCCs) can be a diagnostic challenge based on morphology alone. In this study, we evaluated the diagnostic utility of an anti-vimentin antibody to differentiate RUCs from UCCs.

Materials and Methods: Eighteen calculi patients, 17 urothelial carcinoma patients, and 21 patients without calculi or urothelial carcinoma were examined. Urine cytology slides were prepared using the SurePath method, immunoenzyme stained with the anti-vimentin antibody, and the number of vimentin-positive cell clusters was counted.

Result: In the RUC group, vimentin showed strong staining in 18/18 (100%) cases and 87/202 (43.1%) cell clusters. The UCC group showed positivity for vimentin in 2/17 (11.8%) cases and 2/455 (0.4%) cell clusters. The vimentin-positive rate of RUCs was significantly higher than that of UCCs ($P < 0.001$). In patients without calculi or urothelial carcinoma, vimentin positivity was found in 2/21 (9.5%) cases and 2/938 (0.2%) cell clusters.

Conclusion: The results of our study suggest that immunoenzyme staining of vimentin in urine cytology can help to distinguish RUCs from UCCs.

Disclosure of Interest: None declared.

P-134

Cytological and Immunological Detection of Polyomavirus in Urine Sediment. A Case Report

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Introduction: Polyoma viruses are often not cleared from the body after primary infection and BK and JC strains often remain latent in the transitional cell layer of urinary tract. Latent polyoma virus infections cannot be identified cytologically, histologically or by immunologic detection in cytologic and histologic specimens, but rather require the use of molecular techniques for virus detection in urine and blood. Viral reactivation occurs when the slight changes in the immune system happen and transient reactivation of polyoma virus can be seen in less than one percent of all urine cytology specimens. Cytomorphologically this reactivation is manifested by shadding viral particles inclusions bearing epithelial cells, called decoy cells in urine. Most common are classical decoy cells (type one) characterized by large, homogenous ground glass intranuclear inclusion bodies and condensed rim of chromatin. A higher incidence of decoy cell in urine could be seen in immunocompromised patients, patients suffering from cancer, and patients with allograft transplantation.

Aim: To present a case of patient with decoy cells in urine cytology and previous history of lung cancer.

Case Report: Sixty year old male patient came to regular cheque up complainig to dyspnea and dysuria. A year ago he had lobectomy of the left lung because ot lung tumor and he underwent to chemotherapy and radiotherapy. He also suffers from reumathoid arthritis and chronic prostatitis. Laboratory findings showed severe anemia and erithrocyturia. Cytology findings in urine sediment showed severe atypia of urothelyal cells with intranuclear inclusion (decoy cell type one) and erithrocyturia. Immunocytochemical staining was preformed with monoclonal antibody for polyoma virus antigen, common to all known polyoma virus strains pathogenic in humans, and was positive in nuclei of most cell with decoy cell morphology. Thus, in our patient characteristic cytomorphology and polyoma virus antigen nuclear positivity in urine cells with nuclear atypia refer to polyoma virus infection.

Conclusion: Significant nuclear atypia of decoy cells could be missinterpretaed as cancer cells. Thus, beside decoy cells cytomorphology recognition, immunocytochemical detection of human polyoma virus antigen contibute to cytologic accuracy. In our patient differential diagnosis of metastatic disease or second tumor was exluded with morphology features and nuclear immunopositivity for polyoma virus antigen, confirming role of cytology and immunocytochemistry as simple, qiuck and precise method.

Disclosure of Interest: None declared.

P-135

Significance of Urinary Cytology for Detecting Urothelial Lesions: Three Cases of Flat Carcinoma in situ of the Ureter

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Objectives: We have experienced three cases of flat urothelial carcinoma in situ of the ureter. This condition was not shown in morphological findings of the operative specimens by macroscopic examination. However, the cytological results of all cases were positive for carcinoma not only by microscopic examination but also initially by urinary examination. Our objective is to emphasize the value to routine practice of cytologically examining urine for detecting carcinoma in situ.

Case Reports: The patients were a 78-year-old man, an 84-year-old man, and a 57-year-old man. The patients' voided and catheterized urines were examined cytologically by Papanicolaou stain with routine observations. The stain results were positive for malignant cells. We presumed the patients' lesions to be high grade urothelial carcinoma. For all three patients, nephrectomy and ureterectomy were performed. However, macroscopic examination did not reveal any pathological findings on the mucosa of the ureter or the renal pelvis. Consecutive specimens of the ureter revealed flat urothelial carcinoma in situ in all cases.

Conclusion: Cytological examination of urine is a very useful tool for detecting flat urothelial carcinoma in situ of the ureter. Cases such as the above accentuate the need for skills and procedures that enable early detection of cancer cells by urinary examination.

Disclosure of Interest: None declared.

P-136

Comparative Analysis of Fatal Urinary Bladder Carcinomas and Long-Survived Carcinomas with Multiple Recurrences – Urinary Cytology from the View of Histological Viewpoint

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Objectives: Urinary tract carcinoma recurs frequently, but there are many cases of showing long-term survival without metastasis. Early detection of carcinoma is of little significance, and identification of life-threatening findings is much more valuable. Therefore, to identify the diagnostic features that indicate life-threatening urinary bladder carcinoma, we compared the histological findings in cases of fatal urinary bladder carcinoma (hereinafter the 'cancer death group') and carcinomas that survived for more than 10 years with more than four recurrences (hereinafter the 'survival with recurrence group').

Materials and Methods: Between 1988 and 2014, urinary carcinomatous cytology specimens were submitted and urinary bladder carcinomas were diagnosed histologically without other primary carcinoma in 681 cases. Of these, nine cases were classified in the 'cancer death group' and 14 cases were classified in the 'survival with recurrence group'.

Results: Existence of muscular invasion was high in the 'cancer death group', but other histological characteristics such as the presence of adenocarcinoma or squamous cell carcinoma except transitional cell carcinoma, existence of in situ carcinoma, and atypia of carcinoma cells showed no notable intergroup differences.

Conclusion: The 'cancer death group' did not contain cases of continuous submission of mildly atypical cytology specimens throughout the course, therefore, it is important to diagnose specimens with definite atypical cells.

Disclosure of Interest: None declared.

P-137

A Case of Urothelial Carcinoma with Sarcomatoid Variant of the Urinary Bladder

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Objective: Urothelial carcinoma with sarcomatoid variant of the urinary bladder is a rare and there are only a few analyzed reports of urine cytological features. We present a case of invasive urothelial carcinoma with mesenchymal elements of chondrosarcoma of the urinary bladder.

Material/Case Presentation: A 85-year-old Japanese male, living in a nursing home, presented with gross hematuria, anemia, hypoproteinemia. A year advanced before admitted our Hospital, he had urinary retention and has having a urethral catheterization. Past histories; Dementia, arrhythmia and chronic heart failure since age of 75. Cystoscopy and Computed tomography revealed a multiple pedunculated mass of the urinary bladder and transurethral resection was performed.

Results/Catheterized Urine Cytological Findings: Background was necrotic. There were some large clusters of tumor cells and relatively large amount of dispersed isolated, less cohesive cells were found. Most of the cells of clusters showed high nuclear/cytoplasmic (N/C) ratio, with irregularly arranged oval shaped nuclei. Some clusters had irregular ductal arrangements at the margin. The isolated cells had enlarged oval nuclei with coarse chro-

matin, conspicuous nucleoli and homogeneous light green thick cytoplasm that indicated mesenchymal differentiation. Moreover there were some middle sized, round shaped binuclear cells with demarcated cell borders, contained clear abundant cytoplasm. They were arranged singly and small aggregates, similar to chondrocytes.

Histological Findings: The solid nests were composed of Invasive urothelial carcinoma, G2~3 with ductal differentiation. Tumor had invaded into the muscular layer. In addition, fibrous mesenchymal cells proliferation and Chondrosarcoma areas were observed.

Conclusion: We presented the cytological features of this rare case which as yet are hardly reported though. It was difficult to analyze the sarcomatoid components prior giving histological evaluation. However, Making cytological diagnosis should be contribute anytime and could be given if an adequate specimens are obtain and evaluate the details of each cells. Also it is an important to be aware of radiological findings that might be helpful to speculate about differential diagnosis.

Disclosure of Interest: None declared.

P-138

Metastatic Chondrosarcoma Detected by Urine Cytology

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Background: Chondrosarcoma is a malignant tumor with pure hyaline cartilage differentiation of adulthood and old age. The most common site of involvement are: bones of the pelvis, femur, humerus and ribs. To the best of our knowledge, no previous case report exists documenting the identification of metastatic chondrosarcoma within urine cytology specimens. Herein we report the detection of a case of metastatic chondrosarcoma of the kidney within urine specimen.

Case Report: A woman in her 60s with previous history of chondrosarcoma of the right femur and lung metastases with subsequent segmental lung resection was referred to our institution since left renal mass was detected in the computed tomography during regular clinical follow-up. Under the clinical suspicion of primary renal tumor, void urine cytology and fine needle biopsy of the renal mass were performed. The urine smears reveal neoplastic cells with low N/C ratio, condensed nuclear chromatin with prominent nucleoli and occasional binucleated tumor cells, dispersed within an amorphous myxoid background. The tumor cells are accompanied by inflammatory cells and a few clusters of unremarkable urothelial cells. Moreover, scattered multinucleated cells and cells containing cytoplasmic vacuoles are also identified on liquid-based cytology smears. Microscopically, tumor cells with high N/C ratio with perinuclear halo admixed with binuclear cells within the myxoid matrix are found in the renal biopsy specimen. The cytologic and histopathological features reflected those of the primary tumor and the final diagnosis of metastatic chondrosarcoma of the kidney was reached.

Conclusions: The detection of metastatic chondrosarcoma cells in the urine is extremely rare and requires differential diagnosis with clear cell carcinoma of the kidney and clear urothelial carcinoma. Clues from urine cytology such as the presence of binucleated cells, perinuclear halo, amorphous myxoid background combined with the histopathological features may direct the correct diagnosis.

Disclosure of Interest: None declared.

P-139

Opportunities of Cytological Diagnosis and Prognosis of Prostate Cancer

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Objectives: To represent the opportunities of the contemporary cytological research of material obtained by the transrectal needle biopsy (TNB).

Materials and Methods: Cytological research of the materials obtained by TNB method was performed among the 232 patients with PSA level higher than 4 ng/ml. Prostate cancer (PC) of 178 (76.7%) patients and 54 (23.3%) patients with benign prostatic hyperplasia were cytologically exactly detected using the DNA-FCM data (EPICS-XL, Beckman Coulter, USA and MultiCycle, Phoenix Flow Systems, USA). TNB was performed with Magnum (Bard, USA) 18th calibre needles.

Results: High differentiated adenocarcinoma (HDA) was detected in 54 (23.3%) patients, moderately differentiated adenocarcinoma (MDA) was detected in 71 (39.9%) patients and low differentiated adenocarcinoma (LDA) in 53 (29.8%) patients. In many cases cytological research of TNB was the only morphological method before the therapy starting. In cases when the material was not received in the biopsy material, in cytological specimen there were cells, based on which the diagnosis was formed. For 11 (4.7%) patients cytology diagnosis was the basis for the PC recognition on the preoperative stage. In our research cytological method optimised the morphological diagnostics of PC based on the TNB materials. In case of smear examination – biopsy specimen print – there are no tissue deformations; definition of marks that have key role in PC diagnostic, such as cell modification character, nuclear membrane boards, cells nuclears and their quantity, are more clear. This is also relevant for cases with the lack of biopsy materials or when the cells elements of cancer are get to the prints, given that they are single and could not be evaluated reasonably. The precision of cytological method in PC diagnostics in TNB material is equal to 98.7%. Patients with advanced PC process were defined with the high level of Gleason scale. Patients with HDA in amount of 43 (79.6%) patients were detected with 1–4 score and 11 (20.4%) of patients were detected with 5–7 scores of Gleason scale. In group of 58 (81.7%) patients with MDA Gleason scale score was equal to 5–7 scores and 13 (18.3%) of patients were marked with 1–4 scores of Gleason scale. 8–9 scores of Gleason

scale were defined within 48 (90.5%) patients with LDA and 5–7 scores within 5 (9.4%) patients.

Conclusions: Cytological research and DNA-FCM data provide us with exact prostate cancer diagnosis and prognosis. Cytological research of TNB material was essential and for some cases was the final stage of preoperative prostate cancer diagnostics. As far as prostate cancer morphological differentiation rate declines, we observe the tendency of Gleason scale score increase.

Disclosure of Interest: None declared.

P-140

Enterogenous Cyst of the Testis

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Background: Enterogenous cyst is considered to be a remnant of embryonal developmental anomaly. Commonly, it is found in the mediastinum and in the abdomen. We experienced two cases of enterogenous cyst of the testis with inflammation and disscuss on their cytological feature.

Case 1: A 20-year-old man had felt pain of the left testis and inguinal region two days earlier. Cystic lesion was found and removed. At operation, imprint cytology was performed. In background there were a lot of inflammatory cells including histiocytes and multiple nucleated giant cells. A small cluster consisted of clumnar cells. These cells had round to oval nucleus without atypia. Nucleoli and chromatin were inconspicuous. Some columnar cells with abandant mucin were also noted. Histologically, the cyst with inflammation was covered with columnar epithelia containing mucin surrounded by smooth muscle.

Case 2: A 13-year-old man had felt pain in the left inguinal region. By ultrasound, cystic mass was found and nucleated. Imprint cytology revealed that a small amount of epithelia with mucin in the cytoplasm were found. They did not have cytological atypia. Inflammatory cells including histiocytes were also noted. Histological findings was almost the same as case 1, that is, the cyst was covered with colmunar epithelia with goblet cells surrounded by smooth muscle.

Conclusion: Both of the cases were diagnosed as enterogenous cyst. The differential diagnosis is teratoma and serous cystadenoma. When we meet only columnar epithelia with mucin by cytology, we should remember enterogenous cyst.

Disclosure of Interest: None declared.

P-141

Urine Analysis of School Age Children of Dharan Municipality, Nepal

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Ojectives: To assess the prevalence of urinary abnormalities in school-aged children of Dharan Municipality.

To provide the report of the urine analysis to the students which may help for their further health consciousness.

Methods: Cross sectional, purposive sampling study of 200 urine samples of school age children (less than or equal to 10 years) was done.

Results: 54% and 46% were male and female subjects among which the lowest and highest age was 5 and 10 years respectively. Most common pH finding was 6.0, yellow color followed by straw color and clear white respectively. Highest specific gravity of urine was 1.015 (80%) followed by 1.020 (44%). Ketone bodies and bilirubin positivity were found in single sample each. 7% of urine samples had bacteriuria along with 13 cases showed nitrite positivity. Two urine samples had trace amount, while one showed 1+ amount of glucose.

The frequency of leukocyteuria showed trace, 1+ and plenty amount in nine, three and in one individuals respectively. Hematuria was found to be seen in 8% cases with 2+ in one individual Eight students had positive protein value, of which seven had trace and one had 1+ in amount. One student had plenty of epithelial cells followed by 1+ and trace amount of epithelial cells by two and five students respectively. One had dumble shaped crystals followed by two having each of amorphous, phosphate, calcium oxalate and uric acid crystals respectively. The cross tabulations between the gender with presence of RBCs, WBCs, occurrence of heamaturia and bacteriuria showed significant p-values.

Conclusion: Mass urinary screening proved to be an useful tool to identify children with asymptomatic progressive renal diseases.

Furthermore, the delivery of the report and counseling them for further preventive measures may also help in improving their condition.

Disclosure of Interest: None declared.

Brain

P-142

2,3,7,8-Tetrachlorodibenzo-p-Dioxin Exposure Promotes the Proliferation of Astrocytes via the AKT/STAT3 Pathway: A Role in Astrogliosis

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Objectives: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), one of the environmental persistent organic pollutants. Animal experiments and various epidemiological studies have shown that TCDD exposure could affect the nervous system, but the underlying mechanism has remained unclear. Astrogliosis, a recation of astrocytes seen in a multiple of neurological disorders, has been intensively studied these years. In this study, we aim to investigate the role of TCDD in regulating the proliferation of astrocytes and the possible pathway involved in it.

Materials and Methods: The C6 glioma cells or primary astrocytes were treated with 0, 0.1, 1.0, 10, 50 and 100 nM TCDD for 1 h, 3 h, 6 h, 12 h, 24 h and 48 h. EdU-based proliferation assay and flow cytometry were used to estimate the proliferation of C6 glioma cells. The protein level of Akt, p-Akt, STAT3, p-STAT3, CyclinD1, GD and GFAP were analysed by western blot. Immunofluorescence staining was used to visualize the change of cell morphology and the localization of STAT3 after exposed to TCDD. To inhibit the activity of Akt and STAT3, cells were pretreated with LY294002 and AG490, respectively.

Results: 1 nM, 10 nM and 50 nM TCDD significantly promoted the proliferation of C6 from 12 h, and reach the peak at 24 h. The primary astrocytes showed up-regulation of GFAP and hypertrophy of cellular processes after treated with 10 nM TCDD for 24 h. The level of p-Akt and p-STAT3, which play important roles in the process of astrogliosis were elevated after treated with 10 nM TCDD for 24 h. The downstream molecules, CyclinD1 and GFAP, showed the same trend. And TCDD also promoted STAT3 translocate from cytoplasm to nucleus. Inhibition of Akt and STAT3 reduced the proliferation of C6 and the up-regulation of CyclinD1 and GFAP after TCDD treatment.

Conclusion: TCDD exposure could promote the proliferation of C6 glioma cells and astrocytes via Akt/STAT3 pathway, which may participate in the astrogliosis.

Disclosure of Interest: None declared.

P-143

Intraoperative Squash Cytology of Central Nervous System Lesions: An Analysis Based on 326 Cases

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Introduction: Intraoperative consultation is an important component in the surgical management of CNS lesions. Critical decisions regarding treatment and the extent of surgical aggression can sometimes depend on an appropriate intraoperative histo-pathological diagnosis.

Objective: This study was performed to assess the accuracy and utility of intra-operative consultations for cyto-morphological diagnosis by squash smear technique and their correlation with histo-pathological diagnosis in CNS disorders.

Material and Method: A retrospective study of 326 intra-operative consultations for CNS (304 cranial and 22 spinal) disorders was performed. Squash smears were prepared from the samples sent in isotonic saline for immediate processing and were stained by the Haematoxylin and Eosin method. The cyto-morphological features were noted and correlated with final histo-pathological diagnosis.

Results: Overall concordance between the intra-operative diagnosis and the final diagnosis was seen in 91.10% of cases. The study demonstrated 96.75% accuracy for glial and glio-neuronal tumors and 82.07% for non-glial tumors.

Conclusion: Squash smear technique is a fairly accurate, relatively safe, rapid, simple, easily reproducible, and cost effective tool to diagnose brain tumors. Squash smear cytology is of great value in intra-operative consultation in CNS pathologies.

Disclosure of Interest: None declared.

P-144

Cytological Feature of Epithelioid/Rhabdoid Glioblastoma

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Background: Glioblastoma is a malignant brain tumor originated from glia and devided into various histological subtypes. Epithelioid/rhabdoid glioblastoma is a rather new concept of glioblastoma. This histological feature means more malignant prognosis than other subtypes. We experienced epithelioid/rhabdoid glioblastoma of the frontal lobe, and here, discuss on characteristics of its cytological feature.

Case: A 31-year-old male had been suffered from headache for 3 months. Gradually, gait disorder and appetite loss appeared. He was found lying on the floor with unconsciousness, and transferred to the hospital. Brain CT revealed bilateral tumor of the frontal lobe.

Pathological Findings: Imprint cytological specimen was obtained at operation. Cytologically, large atypical cells were noted sparsely or cohesively. Their cytoplasm was broad and large nucleus located periphery. Its chromatin was coarse granular and nucleolus was inconspicuous. Histologically, there was diffuse proliferation of atypical cells, which had oval to round nuclei and eosinophilic broad cytoplasm. Nuclear inclusion or ICL were also noted. There were many mitoses, hemorrhage and necrosis. Immunohistochemically, vimentin, S-100 protein were positive and GFAP, IDH1, AE1/AE3, CK5/6, and CK20 were negative. Genetical examination showed BRAF mutation. We considered this tumor as epithelioid/rhabdoid glioblastoma.<Conclusion> Histological specimen contained only epithelioid/rhabdoid feature and no conventional glioblastoma in this case. Recently it is reported that epithelioid/rhabdoid glioblastoma shows positivity for vimentin, p53 and INI-1, even if S-100 protein, GFAP are negative. It is not easy to diagnose epithelioid/rhabdoid glioblastoma only by cytologycal examination, however, it may lead to its diagnosis together with cinical information.

Disclosure of Interest: None declared.

P-145

Gliosarcoma with Posterior Mediastinum Metastasis

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Background: Gliosarcoma is an uncommon variant of glioblastoma characterized by a biphasic pattern of glial and mesenchymal differentiation in the tumor. This is the first description of the cytomorphological features of gliosarcoma cells in an extracranial location accompanied by corresponding histopathological findings, clinical, and radiological data.

Case Report: The patient is a 51 year old man who initially presented with a right temporal lobe and internal capsule lesion and subsequently underwent craniotomy with tumor resection and chemoradiation. The pathology diagnosis was gliosarcoma. Three months after surgery, the patient presented with a new posterior mediastinum mass which was aspirated through EUS-guided FNA. Aspirates of gliosarcoma cells appear as a loosely cohesive population of high-grade malignant cells with both pleomorphic epithelioid and spindle cell morphology. Epithelioid cells have eccentric nuclear placement whereas mesenchymal elements have both round cell and sarcomatous morphology. Cells display macronucleoli, bizarre multinucleation and markedly enlarged and bizarre nuclei, cytoplasmic vacuoles and long hair-like cytoplasmic processes. Deep nuclear clefts and occasional prominent nucleoli are also apparent. Mitoses are frequently observed.

Conclusion: The case illustrates the ability of primary cerebral gliosarcoma to involve the posterior mediastinum and that both glial and mesenchymal elements can be aspirated and recognized cytologically.

Disclosure of Interest: None declared.

P-146

Central Neurocytoma Arising in Lateral Ventricle. Report of Two Cases

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Background: Central neurocytoma is a rare neural cell tumor arising in ventricles and its cytological features are not yet clearly understood. We report here cytological feature of two cases with cerebrospinal fluid (CSF) and imprint specimens along with histological findings.

Cases: *(Case 1)* A 33-years old female was pointed out a tumor in left lateral ventricle by CT during treatment of parotitis and meningitis. MRI disclosed round tumor measuring 46x34x42 mm in left lateral ventricle. Tumor was resected by craniotomy and intraoperative diagnosis was central neurocytoma. Residual tumor was treated by Gamma Knife surgery and under following up. *(Case 2)* A 28-years old male noticed an amnesia attack for several years. MRI disclosed round tumor measuring 45x8x44 mm in left lateral ventricle. Tumor was resected by craniotomy and intraoperative diagnosis was central neurocytoma. Residual tumor was treated by radiation therapy and under following up.

Cytological Features: Specimens were prepared from imprint and CSF (case 1) and tumor imprint (case 2) and showed almost same features. A large number of tumor cells were observed with delicate network of small vessels. Tumor cells were uniform in shape and appeared in sheet-like clusters or in alveolar structures with focal rosette formations. Cytoplasms were round and stained clearly or focally reticularly. Numerous small vessels were present inside cell clusters in CSF sediments.

Histological Features: Case 1 and 2 showed almost same features. The tumor was grayish to white in color and hemorrhagic. Tumor cells with lightly eosinophilic cytoplasm and round nuclei were arranged densely. Small vessels were abundant with perivascular fibrillary stroma. Immunostaings were positive for synaptophysin, NeuN, focally positive for S-100, negative for GFAP, NF, Olig2. Case1 was maximal MIB-1 labeling index of was 10.3% for case 1 and 6.9% for case 2 with diagnosis of atypical central neurocytoma for both cases.

Conclusions: Central neurocytoma accounts for only 0.25–0.5% of intracranial tumors. Its cytological features are rarely reported and its findings in CSF was not yet reported. Combination of CSF and imprint specimen along with histological specimen enabled precise observation of its morphological features.

Disclosure of Interest: None declared.

P-147

Cytologic Features of Atypical Meningioma: Based on Examination of Three Cases

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Objective: Meningioma is one of the relatively common tumors and accounts for approximately 20% of primary brain tumors. Meningiomas are histologically classified into grade I to grade III and most meningiomas are grade I. It is important to precisely distinguish grade II and grade III tumors from grade I because of different strategies and outcome. We report some diagnostic cytological features obtained from three cases of atypical meningioma encountered recently.

Materials and Methods: The material consisted of three atypical meningiomas found in 62 meningiomas microscopically confirmed in our hospital between 2013 and 2015. These cytology preparations were routinely processed during frozen section examination for brain tumors in our hospital. We retrospectively examined smear/squash preparation cytology prepared during surgical operations and discussed their findings.

Result: The most diagnostic cytology findings suggestive of atypical meningioma included higher cellularity, spindle-shaped tumor cells with enlarged oval or spindle nuclei, and distinctive nucleoli in addition to conventional meningioma cytology features: whorl formation and intra-nuclear pseudo-inclusions. The nuclei of atypical meningioma showed polymorphism, granular chromatin pattern, and thin membrane. Also noted was loss of cell attachment. There was no tumor necrosis in all cases and mitotic figures were identified in only one case.

Conclusion: Squash preparation cytology was able to distinguish grade II and grade III meningioma from grade I easier than frozen section diagnosis because squash preparation cytology had an advantage to evaluate cellularity and cellular atypia. A combination of squash preparation cytology and frozen section examination may contribute to correct diagnosis of atypical meningioma during surgery.

Disclosure of Interest: None declared.

Oral

P-148

Cytological Characteristics of the Oral Mucosa: Liquid-Based Cytology versus Conventional Cytology

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Objectives: Liquid-based cytology (LBC) was introduced and established a useful method. However, the cytological findings of LBC specimens and conventional (Con) specimens are not entirely same. Here, we performed this comparison for the aim to determine the cytological characteristics of LBC methods compared with conventional smear methods in scraping samples of the oral mucosal epithelium.

Materials and Methods: This study was conducted in Asahi University, School of Dentistry. Total of 25 cases were studied, which were distributed 14 cases of normal tongue epithelium and 9 cases of oral epithelial lesions (hyperkeratosis, epithelial dysplasia and squamous cell carcinoma). In each case, twice scrapings were performed. The first scraping was for Con preparation and the second scraping yielded material for LBC preparation. Both were analized in nuclear and cytoplasm area size, and tint of cytoplasm by WinROOF photometric analyzing system.

Result: In cellular and nuclear area size ratio, both OG and LG cells shrunk approximately 30% by the LBC than Con ($p < 0.001$). In N/C ratio, both OG and LG cells showed slightly higher by LBC than Con (There is no significant difference).

Conclusion: With regard to clear background, monolayer cell preparation and cell preservation, LBC preparations are superior to Con specimens. LBC preparation is easy to decrease of time consuming and preparation step for screening and interpreting because the cells are located in the limited area with clear backgrounds, and appropriate for excellent cellular preservation. However, LBC preparation is more expensive than Con and require some experience for interpretation of findings. The LBC method of oral mucosa can become the useful technique equivalent to the Con method by understanding the cytological characteristics.

Disclosure of Interest: None declared.

P-149

Qualitative Difference between Conventional Method and LBC Method in Oral Squamous Cell Carcinoma

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Objectives: Study on the difference between conventional and LBC method in the oral cavity area is few, and is not performed particularly qualitative study. We examined qualitative difference between conventional and LBC in oral squamous cell carcinoma (brightness, edge irregular degree, intranuclear distribution).

Materials and Methods: Conventional and LBC slides were prepared that have been diagnosed with oral squamous cell carcinoma in tissue diagnosis. Sites of lesion formation were buccal mucosa, gums, mouth floor. After preparing conventional slides, residual cells in the collecting device were fixed to CytoRich RED (BD) and LBC slides were made according to the manufacturer protocol. Qualitative analysis was performed using ImageJ, analysis item were using histogram mean value (brightness), solidity value (edge irregular degree) and histogram StdDev value (intranuclear non-uniformity).

Result: Results of comparison of 10 samples oral squamous cell carcinoma, higher brightness towards the conventional method than LBC, edge irregular degree and intranuclear non-uniformity were vary greatly towards the LBC method.

Disclosure of Interest: None declared.

P-150

Comparison between Exfoliative Cytology and Histopathology in Detecting Oral Squamous Cell Carcinoma

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Objectives: The purpose of this study is to evaluate the efficiency oral exfoliative cytology in detecting oral squamous cell carcinoma.

Materials and Methods: With the consent of the patients, scrapings were made with wooden spatula from 45 patients with clinically suspicious of oral squamous cell carcinoma. The scrapings were smeared on cleaned glass slides, fixed immediately in 95% ethanol. Then staining with PAP stain and examined microscopically. The biopsies were also taken for all these cases for histopathological confirmation and comparison of results.

Results: Sample profile of 45 subjects, 37 (82.3%) were males and 8 (17.7%) were females with a male to female ratio of 4:1. The majority of subjects (84.6%) were in the age category of 41–80 years. Snuff dipping and alcohol were prevalent among 45 subjects (46.7%), while 5 (11.1%) of them were smokers. many of the lesions 17 (37.8) were diagnosed on the Labiogingiva of the oral cavity. A definite histopathological diagnosis was achieved by conventional incisional biopsy; histopathological diagnoses of two cases of verrucous carcinoma, four cases of hyperkeratosis 31 (83.8%) cases of squamous cell carcinoma sample anatomic profile and habits of study group were compared for their histological diagnoses. A total of 31 (83.8%) cases were histopathologically diagnosed as squamous cell carcinoma (SCC). In cytology, a specificity of 100%, sensitivity of 91.8% were obtained for OSCC. In 37 cases of squamous cell carcinoma 34 patients were cytologically positive and Histopathologically also positive and 3 patients were cytologically negative and histopathologically positive with the sensitivity of 91.8% and specificity of 100%.

Conclusion: Despite the small number of cases in this study. We realized again not only that the diagnostic accuracy of preoperative buccal smear cytology for oral lesion was high, but also that it was a safe, easy-to-perform, clinically very useful diagnostic procedure. Oralexfoliative cytology is simple, rapid, less invasive and relatively painless method, well accepted by patients, and therefore, suitable for population screening program.

Disclosure of Interest: None declared.

P-151

Utility of Fine Needle Aspiration Biopsy in the Diagnosis of Intraoral Lesions

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Objectives: Use of fine needle aspiration (FNA) for intraoral lesions have been limited due to its technical difficulty and preference for performing biopsy by clinicians. We aim to evaluate the diagnostic accuracy of the FNA and proposed the epidemiology related to anatomic zonal distributions of the intraoral lesions.

Materials and Methods: Cytological materials, their histopathological correlates and relevant clinical data of FNA cases that were performed on intraoral lesions during 2007 to 2015 at Chiang Mai University Hospital were collected. A diagnostic test was used to evaluate the accuracy of intraoral FNA.

Results: A total of 78 cases of FNA were performed in intraoral lesions of which characteristics included 42 submucosal (53.84%), 25 exophytic (32.05%), 7 cystic lesions (8.97%) and 4 others (5.14%). Their sizes ranged from 0.5 to 7 cm (2.86 ± 1.49 cm) in the greatest dimension. Their anatomic zones distributed into 22 anterolateral (28.20%), 18 inferocentral (23.08%) and 38 superoposterior (48.72%) parts. Benign cyst was the commonest nonneoplastic lesion, while squamous cell carcinoma was the most common malignant lesion in all regions. Pleomorphic adenoma

was the most common benign tumor which was located in superoposterior region. The overall diagnostic accuracy of intraoral FNA were 56.25% sensitivity, 95.65% specificity, 90% positive predictive value and 75.86% negative predictive value. The likelihood ratio of being malignant outcome when diagnosed by FNA was 12.93 (3.22–51.91). There were 19 (24.26%) cases with unsatisfactory aspirates which were more common in superoposterior zone (10, 52.63%). Histopathological follow-up of these cases were 12 benign lesions (63.16%) and 7 malignant lesions (36.84%).

Conclusions: FNA was useful for evaluation of intraoral lesions. Although its sensitivity was relatively low, high specificity and positive predictive value has supported its application in these regions. Caution should be taken in cases with unsatisfactory cases and must not be treated as negative, since the malignant follow-up were encountered. Proposed three anatomical zones may be helpful for interpretation of FNA materials from these intraoral lesions.

Disclosure of Interest: None declared.

P-152

Fundamental Studies of Cytology by Using a Model Animal

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Objectives: In the treatment and prognosis of oral cancer is early treatment due to early detection is extremely important, and this is one of the important issues to be addressed by the dentist to deal with oral mucosal disease. But although precancerous lesions of oral mucous membrane diseases at present, are the used tissue diagnosis because it is difficult to differentiate only by macroscopic findings (biopsy), which is generally dentistry in that it requires skill and experience it is impossible in medicine. So, as we help early diagnosis determination of oral squamous cell carcinoma and oral mucosal disease, we are actively recommended the introduction of cytology of the oral cavity. Currently, in other organs including the uterine cancer, Pap classification has been used conventionally to vary Bethesda classification is used. There is a need to determine the reason the basis of key diagnostic in performing cytology of the oral cavity 'cells only guidelines', it is also necessary to find a typical example. Therefore, an object of this time, we administered a carcinogenic agent in rats underwent cytology of the oral cavity by using a pre-cancerous lesions and squamous cell carcinoma of the animal model, four stages is a new diagnostic criteria, we would like to determine.

Materials and Methods: I administered as drinking water to carcinogenic agents 4NQO the 6-week-old rats. After 15 weeks of age, which was continuously administered to, the enforcement of brushing cytology by using, for example, interdental brush Check the tongue mucosa disease. Then, the collected cells and tissues, cytology of staining by the conventional method and the LBC method, is observed by performing a Pap staining specimens and IHC staining specimens.

Results: In IHC staining, hnRNPK in the low malignant cells were expressed localized in the cytoplasm, the migration is expressed from the cytoplasm as the grade increases to the nucleus.

Evaluated hnRNPK expression was localized to the cytoplasm of low atypia cells in all layers. In addition, nuclei of the keratinized and/or non-keratinized superficial to middle layer squamous epithelial high malignant cells were stained in hnRNPK.

Conclusion: Cytology is a well established as useful method in the screening whether mucosal malignant or pre-malignant lesions. Also, rather than diagnosis only Pap staining, that will be used in combination also IHC staining, it is believed that the help of a more accurate diagnosis. By going further performing a search in gene expression, it seems to be able to better present the utility of cytology.

Disclosure of Interest: None declared.

Salivary Glands

P-153

Adequacy and Accuracy of Parotid Gland Fine Needle Aspiration Cytology with Histopathology Correlation: A Five Year Study from a Tertiary Care Centre of India

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Objectives: Fine needle aspiration cytology (FNAC) is an important tool to evaluate and differentiate neoplastic and non-neoplastic lesions of parotid gland and thus provides important preoperative information. We aimed to analyse aspirates of parotid swellings of last five years at our institute. The cases on which specific cytological diagnosis could not be provided were further characterized and correlated with corresponding histopathology.

Materials and Methods: All aspirates of parotid swellings during 2009–2014 were analysed. The cases which were diagnosed as poorly differentiated carcinoma and cases which showed presence of atypical cells and categorised as inconclusive were further studied. These cases were then reviewed and categorised into 5 groups – 'Adenocarcinoma like' group, 'Adenoid cystic carcinoma/Ca ex pleomorphic adenoma like' group, 'Mucoepidermoid carcinoma/ Squamous cell carcinoma like' group, 'Acinic cell carcinoma like' group, and 'Others'.

Results: Out of the 893 aspirates 16%, 12%, 51% were positive, inconclusive and negative for malignancy and unsatisfactory respectively. Commonest benign and malignant tumour were pleomorphic adenoma and mucoepidermoid carcinoma respectively.

Out of 105 cases which were selected for further analysis, 35 cases had corresponding histology and were reviewed and classified into the 5 groups. 'Adenocarcinoma like' and 'Mucoepidermoid like' were commonest subgroups on review of inconclusive cases.

Conclusion: The diagnosis of benign and malignant tumour could be offered preoperatively on FNAC with a low false positive rate, assisting appropriate surgical management. Accurate subtyping was possible in many cases however immunocytochemical evaluation may be needed in cases with equivocal morphology on cytology.

Disclosure of Interest: None declared.

P-154

Fnac Study of Adenoid Cystic Carcinoma of Various Sites

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Objectives:

1) To study cytomorphological patterns of adenoid cystic carcinoma (AdCC) of various sites.

2) Correlate hematoxylin and eosin stained slides with Giemsa stained smears.

Materials and Methods: This is a retrospective study of 4 cases who were diagnosed as adenoid cystic carcinoma of various sites by pre-operative fine needle aspiration cytology in Goa Medical College from January 2005 to December 2015.

Results: The study comprised of 2 males and 2 females. Mean age was 56 years. FNAC of the following sites was performed; trachea, submandibular salivary gland, uterine cervix and maxillary sinus. FNA smears were made and stained with H&E and Giemsa. A diagnosis of adenoid cystic carcinoma was made. There was complete agreement between needle biopsy and surgical histology specimen in 3 out of 4 cases. The 4th case, i.e. of the submandibular salivary gland has not been operated yet.

Conclusions: Pre-operative cytological diagnosis of AdCC is important not only due to excellent prognosis, but also because it may play a role in determining treatment.

Disclosure of Interest: None declared.

P-155

Low-Grade Mucoepidermoid Carcinoma with Prominent Tumor-Associated Lymphoid Proliferation of Parotid Gland: A Diagnostic Challenge

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Background: Mucoepidermoid carcinoma (MEC) is the most common primary malignant neoplasm of the salivary gland. We herein report the case of MEC displaying prominent tumor-associated lymphoid proliferation (TALP) together with multiple cystic spaces, mimicking a lymphoepithelial cyst.

Case Report: A-38-year-old female patient with a previous history of papillary carcinoma of thyroid gland, presented with a painless mass at the right parotid region. This lesion had appeared 2 months before medical attention and was felt to be slowly growing. On physical examination, the mass was mobile and firm. A preoperative fine needle aspiration (FNA) was performed revealing trabecular, solid, and cribriform sheet of basaloid epithelial cells within lymphocyte-rich proteinaceous background. A cytological diagnosis on FNA material was 'basaloid cell tumor, favor adenoid cystic carcinoma.' On surgical examination, the tumor was not fixed to adjacent structures. The patient underwent excisional biopsy and macroscopic examination revealed a circumscribed lobular mass with solid-cystic appearance composed of white-tan tissue with cystic spaces up to 0.5 cm containing thick yellow-gray mucous material. Intraoperative frozen section was reported as lymphoepithelial cyst-like lesion with a 0.5 cm area which was suspicious of invasive epithelial growth. Microscopically, it consisted of multiple intracystic papillary projections lined by basaloid or intermediate cells. The solid part was composed of irregular shaped sheets of squamoid cells admixed with goblet cells. Interestingly, the stroma also displayed prominent reactive lymphoid tissue. No evidence of cellular anaplasia, mitotic figure, necrosis, and vascular or perineural invasion was observed. The final pathological diagnosis was low-grade MEC with TALP. In this case, a complete surgical excision and long term clinical follow-up are an adequate management.

Conclusion: This case demonstrated an aspect of differential diagnoses on the cytological material that were composed of basaloid cells within rich lymphoid background.

Disclosure of Interest: None declared.

P-156

Cystic Lesions of the Parotid Glands

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Objectives: Fine-needle aspiration (FNA) play an important role in the evaluation of parotid gland swellings. However, a variety of the parotid glands lesions have a predominantly cystic architecture. We aimed to analyze the correlation between cystic contents lacking of epithelial cell components and the final histology results.

Materials and Methods: We reviewed the cytologic materials of 164 patients with parotid gland swellings who had undergone surgery at our Hospital between January 2010 and March 2012. FNAC were carried out on parotid gland lesions without ultrasound guidance. The findings of FNA were compared with histological diagnosis in these cases.

Results: Aspirates of 13 lesions yielded watery or mucoid material or blood only and epithelial cellular components was absent. Histopathological diagnosis of these lesions were benign cysts, inflammatory affection, Warthin's tumors, pleomorphic adenomas, mucoepidermoid carcinomas, MALT lymphoma, acinic cell carcinoma, basal cell adenoma, metastatic papillary thyroid carcinoma, respectively.

Conclusion: Aspirates of neoplastic and nonneoplastic lesions of the parotid glands may both contain no epithelial cells and result in false-negative diagnoses. Therefore, we recommend repeat aspiration with ultrasound guidance in cyst contents only cases.

Disclosure of Interest: None declared.

P-157

Mammary Analogue Secretory Carcinoma of the Parotid Gland: A Case Report

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Background: A mammary analogue secretory carcinoma (MASC) is a newly reported tumor entity of salivary glands, but some of them might have been labeled as acinic cell carcinoma. We report a case of MASC in the parotid gland.

Case: A 50-year-old man developed the right face paralysis. Fine needle aspiration cytology and biopsy were performed on the right parotid gland and it was reported as 'acinic cell carcinoma' in other hospital. Magnetic Resonance Imaging showed a mass in the right parotid gland deep layer and the parapharyngeal space. There was no metastasis in the brain. A right parotidectomy and lymphadenectomy were performed. The patient is alive with disease three months after surgery.

Imprint Cytologic Findings: It was cellular smear with sheet-like or loosely cohesive clusters of atypical cells in the background of lymphocytes. The cells had eccentrically located round to oval nuclei, prominent nucleoli and foamy or glanular abundant cytoplasm. Intracytoplasmic lumina (ICL) or mucous globular structures (MGS) in cytoplasm were observed. Initially acinic cell carcinoma was suspected.

Macroscopic Findings: An infiltrating white and soft tumor measuring 40x35 mm.

Microscopic Findings: The tumor was characterized with alveolar, glandular or cribriform arrangements of round cells with round or oval nuclei and prominent nucleoli. Signet-ring cells or round cell with cytoplasmic vacuoles containing secretory pale eosinophilic materials were observed. The stroma was fibrous with hyalinization. The secretory material was diastase-resistant PAS positive, but cytoplasm was negative for PAS stain. Immunohistochemically, the tumor was positive for S100, CAM5.2, EMA, vimentin, mammaglobin and GCDFP 15 and negative for SMA and p63. PCR and FISH analyses detected ETV6-NTRK3 fusion gene transcript. There was no lymph node metastasis.

Conclusions: We made a final diagnosis of MASC in histology. The differential diagnosis between acinic cell carcinoma and MASC is very difficult in cytology. The presence of ICL or MGS is a diagnostic clue of MASC. It is recommended that careful cytological diagnosis is essential, taking MASC into consideration, in 'odd looking acinic cell carinomas'.

Disclosure of Interest: None declared.

P-158

Cytological Variations and Differential Diagnosis of Mammary Analogue Secretory Carcinoma (MASC)

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Objectives: Mammary analogue secretory carcinoma (MASC) is a recently described salivary gland neoplasm that is defined by *ETV6-NTRK3* gene fusion. In MASC, wide varieties of cytological findings have been reported.

Materials and Methods: We examined the clinicopathological and cytological features of 14 cases of MASC defined by RT-PCR and/or FISH analysis of the *ETV6-NTRK3* fusion gene.

Results: The cases occurred in 6 men and 7 women aged between 39 and 68 years, with a mean of 47.3 years. In 11 of these 14 cases, the tumor involved the parotid gland, the submandibular gland in two and the accessary parotid gland in one case. Twelve cases were in Stage I/II, but two cases were in Stage IV.

Histologically, twelve cases showed mixed patterns of microcystic and/or follicular and/or papillary-cystic and/or solid structures. All tumors were immunoreactive for mammaglobin, S-100 protein and vimentin. Available fine-needle aspiration cytology smears were cellular and exhibited many loosely cohesive syncytial clusters or isolated cells. Many histiocytes, some of which contained hemosiderin pigments and variously shaped mucinous material were evident in the background or within the epithelial clusters. Rarely necrotic cells were found. The majority of cases showed small to

medium-sized follicular structures with secreted materials. Papillary clusters were occasionally found. In one case, small acinar clusters were found. Tumor cells exhibited small to medium-sized round to oval nuclei, with a smooth contour and indistinct or small nucleoli, and vacuolated cytoplasm. Binuclear cells and plasma cell-like cells with eccentric nuclei were often seen. In some cases tumor cells had enlarged nucleoli and eosinophilic polygonal cytoplasm. No tumor cells had obvious intracytoplasmic zymogen granules. Differential diagnosis was acinic cell carcinoma, pleomorphic adenoma, myoepithelioma, sebaceous tumor, mucoepidermoid carcinoma, oncocytic tumors, polymorphous low grade adenocarcinoma and low grade cribriform cystadenocarcinoma.

Conclusion: It appeared that clusters of small to medium-sized follicular and papillary configurations consisting of bland tumor cells with vacuolated cytoplasm, but lack of intracytoplasmic zymogen granules, in a mucinous or hemosiderin-laden histiocyte-rich background, were a characteristic cytological feature highly suggestive of MASC. However, differential diagnosis from various other tumors should be considered according to cytological variations in MASC.

Disclosure of Interest: None declared.

P-159

A Case of Mammary Analogue Secretory Carcinoma of Salivary Gland: Cytological Diagnosis

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Objective: We report a case of mammary analogue secretory carcinoma (MASC) of salivary gland with cytological findings. This disease category has been proposed recently, and there are very few descriptions of cytological findings.

Material and Method: A 37-year-old male patient had swelling of the right parotid gland with pain. In suspect of brachiogenic cyst or Warthin tumor, the clinician performed fine-needle aspiration cytology/imprint cytology examination and resection of the tumor of the right parotid gland.

Result: Cytological examination revealed clusters of slightly atypical epithelial cells with faintly eosinophilic cytoplasms with secretary granules, neutrophils, lymphocytes, histiocytes and red blood cells. Partially resected parotid gland was 4x3.5x2.7 cm in size, had a 2x1.5x1.5 cm cyst. Microscopically, small nests of slightly atypical cells infiltrated to the cyst wall. Immunohistochemically, tumor cells showed positive for AE1/AE3, CK-HMW, GFAP, S-100, vimentin, and negative for smooth muscle actin and p63. Labeling index of Ki-67 was <20%. With histological, cytological and immunohistochemical findings, MASC of salivary gland was diagnosed. Rearrangement of ETV6 gene confirmed this diagnosis.

Conclusion: There will be no key points to make a diagnosis of MASC, and careful observation is necessary for cytological diagnosis of this disease.

Disclosure of Interest: None declared.

P-160

Re-Evaluation of Cytology Specimens Obtained from Carcinoma Ex Pleomorphic Adenoma – Trial to Detect Early Signs of Salivary Duct Carcinoma

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Objective: Pleomorphic adenoma (PA) is the common benign salivary gland tumors and it is known that malignancy including salivary duct carcinoma (SDC) may develop after long period. SDC is considered to be a counterpart of ductal carcinoma of the mammary gland. Yet its tumorigenesis process is not clearly understood. In some cases, SDC were developed as 'de novo' lesion like its mammary counterpart. On the other hand, pleomorphic adenoma related SDC are reported. In addition, some Carcinoma ex PA (CXPA), including SDC, revealed abrupt and rapid progress. The amounts of PA and carcinoma components are various so that to detect early lesion, finding specific figures by cytological examination is effective and useful for the treatment.

Materials and Methods: We have experienced two cases of CXPA. Both cases had a long history of parotid mass that had not changed so much in several years. Patients noticed recent enlargement of tumor and the diagnosis of malignancy was considered clinically. Pre-surgical cytological diagnosis was PA (Case 1) and PA with malignancy not otherwise specified (Case 2). In addition, Case 1 revealed extremely rapid growth of tumor after cytological examination, and stamp cytology at the time of excision revealed malignancy. Histological examination revealed that malignant components of Case 1 were SDC, undifferentiated carcinoma and chondrosarcoma. The Case 2 revealed SDC as the malignant component. Histologically, single or small number of SDC cells is noticed in PA region in both cases. Immunohistochemically, SDC cells can be identified by Androgen receptor and/or HER2 stain. We have re-evaluated the previous cytology specimens to detect the signs of SDC.

Result: After re-evaluation of pre-surgical cytology specimens, there are figures of myoepithelial cells intermingled with a few large atypical cells, individually or atypical cells forming small clusters/nests. We considered that this finding suggests early stage of SDC arising from PA. In addition, some dissociated relatively large cells are seen around PA component in the Case 1, which suggest malignancy.

Conclusion: We have concluded that the cytological figure of large atypical cell(s) intermingled with myoepithelial cells and/or myxoid component is cytological counterpart of histologically identified early SDC lesion in PA. When we perform cytological examination for clinically diagnosed PA lesion, especially when they grow fast, we have to pay attention to such delicate findings easily masked by ordinary PA findings, and dissociated larger non-myoepithelial cells.

Disclosure of Interest: None declared.

P-161

Carcinoma (Myoepithelial Carcinoma) Ex Pleomorphic Adenoma of the Palate: A Case Report with Cytologic and Histopathologic Dilemma

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Objectives: Malignant transformation from pleomorphic adenoma (PA) is a rare salivary gland neoplasm formerly called malignant mixed tumor including carcinoma ex pleomorphic adenoma (CXPA), metastasizing pleomorphic adenoma and carcinosarcoma/sarcomatoid carcinoma, however they are independent each other in the present. CXPA is carcinoma component developed from PA, and more categorized into the three types, according to the kind of carcinoma cells invasion i.e. intraductal carcinoma, intra-capsular carcinoma and extra-capsular carcinoma depends on the presence of tumor cells infiltration. Here we present a case of low-grade myoepithelial carcinoma developed from PA and its difficulty of initial cytology and small biopsy excluded PA component.

Materials and Methods: A 83-year-old woman referred to Asahi University Hospital with a chief complain of the ulcerative swelling mass (30 × 30 mm) of the left side palate. There was a rapid increase in size for the past three months in a swelling without significant symptoms presented for over three years. Contrast-enhanced CT image showed defined rounded mass but irregular component inside. Initial fine needle aspiration cytology (FNAC) was performed and smears were fixed in 95% ethanol and stained with Papanicolaou staining. Biopsy was also performed at the same time.

Results: FNAC showed relative cohesive clusters of plasmacytoid and oval-shaped myoepithelial component with increasing chromatin and high nuclear/cytoplasmic ratio in the mucinous fluid background. The biopsy revealed alveolar and strand growth pattern composed of plasmacytoid, epithelioid or spindle-shaped tumor cells with hiperchromatism. Histopathological diagnosis was low-grade myoepithelial carcinoma but the possible malignant transformation occurred in a background of PA was suggested because of the ulcer formation during rapid growth. Radical resection of the palate mass was performed and confirmed the presence of ancient PA. Immunohistochemical staining for the carcinoma cells were positive for CK14, S100, α-SMA, HHF35 and p63, but Ki-67 was not significant (MIB-1 index <10%).

Conclusion: Although it is difficult to diagnose this entity on cytology or small biopsy, CXPA should be suspected when atypical characteristics and clinical information are observed. In rare cases of myoepithelial carcinoma in a preexisting PA, multiple needle passes from different areas of swelling is required for an accurate interpretation.

Disclosure of Interest: None declared.

Thyroid

P-162

Cytohistologic Correlation of Thyroid Lesions: A Six Year Review at the Medical City, Philippines

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Objectives: Fine needle aspiration is proven to be a reliable diagnostic tool in the management of thyroid disorders. It is the aim of this study to assess the accuracy of thyroid cytology performed in a tertiary private hospital and to analyze the discordant cases.

Materials and Methods: 448 thyroid aspirates from 2010–2015 were reviewed and correlated with histodiagnosis. Sensitivity, specificity, positive and negative predictive values, and accuracy were determined. False positive and false negative diagnoses were analyzed.

Results: 326 (73%) were concordant and 122 (27%) were discordant. Sensitivity, specificity, positive and negative predictive values were 58.5%, 83.0%, 71.4%, and 73.5%, respectively. Accuracy was 72.8%. False positive and false negative diagnoses were 44 (36%) and 78 (64%), respectively. Pitfalls in false positive diagnoses included overlapping cytologic features of colloid adenomatous goiter and papillary carcinoma notably in moderately cellular smears with abundant monolayers, branching papillae, and clusters of follicular cells and in mildly cellular smears with focal follicular cell atypia and small cell clusters mimicking the microfollicular pattern of follicular neoplasms. Majority (85%) of false negative diagnoses were due to sampling errors attributable to dual pathology with dominant benign lesion usually colloid adenomatous goiter and papillary microcarcinoma. The small minority (15%) were secondary to errors of interpretation.

Conclusion: Fine needle aspiration cytology of the thyroid requires assessment of overall cellularity, architectural pattern, cellular features, and background components for optimum cytodiagnosis. Adequate sampling, awareness of overlapping criteria in certain thyroid lesions and presence of dual thyroid pathology should also be taken into consideration.

Disclosure of Interest: None declared.

P-163

Diagnostic Efficacy of Liquid-Based Preparation Cytology in Thyroid Fine Needle Aspiration: A Meta-Analysis

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Objectives: For the past decades, fine needle aspiration cytology (FNAC) using conventional smear (CS) has been successfully established as the test of choice for making a diagnosis for thyroid lesions. Recently, liquid-based preparation (LBP) technique has been gaining its acceptance in thyroid FNAC to overcome limitations of CS. However, the results of the accuracy of LBP methods over CS still remain controversial. This study aims to draw a true value of sensitivity and specificity of LBP in thyroid FNAC and compare the diagnostic efficacy with that of CS by meta-analysis.

Materials and Methods: We searched major electronic database (MEDLINE, Embase, Cochrane library, Google scholar) with queries of 'thyroid', 'liquid-based preparation', and 'liquid-based cytology'. Original articles including cytohistologic correlation data comparing the accuracy of any LBP technique (ThinPrep (TP), SurePath (SP)) and CS have been included for qualitative meta-analysis and for drawing sROC curve.

Results: A total of 372 studies were screened and 69 original articles were eligible for full-text reviewing. 21 TP and 6 SP studies were eligible for qualitative meta-analysis, respectively. Sample adequacy was significantly superior in TP than CS (76.0% vs. 66.6%, $p < 0.000001$), and significantly superior in SP than CS (92.9% vs. 86.8%, $p < 0.0198$). Sample inadequacy has been decreased according to the publication year of the studies. In regards to the sampling methods, TP was significantly superior in double sampling, direct to vial, syringe rinsing and CS was superior in consultation slide, sample splitting. sROC curve showed similar results between TP and CS and between SP and CS.

Conclusion: This study showed superior sample adequacy of LBP than CS and similar diagnostic efficacy between LBP and CS in thyroid FNAC. However, the cytomorphologic differences between LBP and CS should be carefully considered.

Disclosure of Interest: None declared.

P-164

Incidence and Malignancy Rates of FNA Diagnoses Using the Bethesda System for Reporting Thyroid Cytopathology: An Institutional Experience

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Objectives: The BSRTC was developed in 2008 as a response to the need for an International and Universal terminology for reporting thyroid cytopathology. It comprises 6 categories with the associated risk for malignancy. The implementation in different institutions however, suffers from controversies regarding incidence and malignancy rates. In our study we will analyze and discuss the experience of our institution on the diagnostic accuracy from the implementation of BSRTC.

Materials and Methods: 610 patients underwent US Guided FNA of a total of 750 thyroid nodules by the experienced in the field Cytopathologist. The cytopathology reports were classified prospectively using the BSRTC criteria. Follow up of patients was based on clinical and sonographic examinations for at least 18 months, additional FNAs or thyroid surgery with the corresponding histopathology report.

Results: The diagnostic incidences for categories I-VI were 5.2%, 82.8%, 5.2%, 1.46%, 1.06%, 5.7% and the corresponding malignancy rates were 5.13%, 1.77% (0–3%), 28.2% (5–15%), 27.2% (12–32%) and 100% for categories V (60–75%) and VI (97–99%) respectively. The anticipated malignancy rates according to the BSRTC guidelines are seen in brackets. In general, there is a great deal of agreement in the implementation of the TBS in our institution. Disagreement is seen in the categories III and V, possibly reflecting the big concern of Greek patients about false positive results and the reluctance of the Cytopathologist to overdiagnose FNAs. Comparison of our results with previous studies show differences that depend on whether the data reflect the general population or can be attributed to reporting practice, patients' management issues and technical reasons.

Conclusion: The BSRTC is an informative reporting system especially for the negative and suspicious/positive categories. The value of BSRTC in proper management of thyroid nodules could be enhanced by the annual report of incidences and corresponding malignancy rates of individual laboratories, especially for the indeterminate categories.

Disclosure of Interest: None declared.

P-165

Histopathologic Correlation of Fine Needle Aspiration Biopsies Reported as Suspicious for Malignancy and Malignant According to the Bethesda System for Reporting Thyroid Cytopathology

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Objectives: Thyroid nodules are common and 5–10% of thyroid fine needle aspirations (FNA) were reported as suspicious for malignancy (SFM) or malignant (M) in the literature. The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) implies malignancy risks as 60–75% and 97–99% respectively and reported malignancy ratios are within the limits of this range in the literature. However occasionally benign nodules are interpreted as SFM/M. There are well defined conditions such as chronic lymphocytic thyroiditis (CLT), oncocytic change, cystic degeneration and worrisome atypia in regenerative/degenerative cells that will cause interpretation errors. Besides these well-known diagnostic pitfalls, 'hyaline degeneration' can also contribute to discrepancy in some of the benign nodules reported as SFM/M.

Materials and Methods: Thirty nine thyroid FNAs classified as SFM/M with subsequent surgical pathology follow-up were identified between 2013 and 2014. Oncocytic change, chronic lymphocytic thyroiditis and cystic degeneration were compared between M and SFM cases. Slides of discrepant cases were reevaluated for the presence of nuclear features of PTC, consistency of colloid and hyaline degeneration.

Results: The study cohort consists of 14 SFM and 25 M cases with 6 males and 35 females. 28.6% of the cases (4/14) within the SFM category were found to be benign, whereas all of the cases in the M category were malignant in the SPFU. With an exception of one medullary thyroid carcinoma, all of the malignant diagnoses were PTC. Benign diagnoses were as follows: adenomatous nodule with oncocytic change, lymphocytic thyroiditis, follicular nodular disease and follicular nodular disease with lymphocytic thyroiditis. Oncocytic change, chronic lymphocytic thyroiditis and cystic change was observed as 64% vs. 57.1%, 16% vs. 28.6% and 0% vs. 21.4% within the M and SFM categories respectively. In the discrepant cases there were no true papillary formations as well as fully developed PTC nuclear features. Except intranuclear pseudoinclusions nuclear PTC features were encountered in the smears to some extent. Prominent hyaline degeneration was found to accompany in three cases where the PTC features were striking.

Conclusions: In this study malignancy ratios of SFM and M categories were found to be relevant with the implied risks of TBSRTC. Although the number of cases was limited, similar to literature; CLT, oncocytic change and cystic change was observed to cause PTC like features in benign thyroid lesions. In addition to these well-known conditions 'hyaline degeneration' could be a possible contributing factor for overdiagnosis.

Disclosure of Interest: None declared.

P-166

International Comparison of Follicular-Patterned Lesions among the Bethesda, the Japanese and the New Italian Systems for Reporting Thyroid Cytology

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Objectives: Standardization of thyroid reporting systems (TRS) is becoming the standard of care for the management of patients with thyroid nodules. Unfortunately, a unique TRS is not available and different national cytological societies have developed their own different TRS. We present a double-blind study of histologically confirmed follicular-patterned thyroid neoplasms and evaluate the performance of different TRS.

Materials and Methods: Twenty consecutive resected thyroid follicular patterned lesions with a preoperative diagnostic FNA were retrieved from the archives of the Ito Hospital, Tokyo, Japan. The fine-needle aspiration (FNA) slide sets were then sent for evaluation to six cytopathologists using the Bethesda, the Japanese and the new Italian TRS (two cytopathologists for both TRS). All FNA cases were classified without aid of clinical information using this 3 TRS. Inter-observer variability was assessed using Cohen's Kappa (K) coefficient. Thereafter, diagnostic performance of each TRS was determined.

Results: The histology of the 20 cases comprised nodular hyperplasia (1 case), follicular adenomas (12 cases), follicular carcinomas (6 cases), and papillary carcinoma, follicular variant (1 case). Diagnostic concordance between each cytopathologists using the same TRS was fair (k = 0.40) for the Italian TRS and the Bethesda TRS (k = 0.38) and very weak (k = –0.07) for the Japanese TRS. Considering only the most experienced cytopathologist in each TRS, diagnostic performance was computed for each TRS and resulted in the following sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy:

Thyroid Reporting System Sensitivity Specificity PPV NPV Accuracy: Bethesda 0.71 0.30 0.36 0.67 0.51.

Italian 0.86 0.61 0.54 0.89 0.74.

Japanese 0.43 0.85 0.60 0.73 0.64.

Conclusion: The different TRS studied showed a wild variation of inter-observer agreement and performance. The goal of a common and comparable TRS is far to be achieved.

Disclosure of Interest: None declared.

P-167

Is the JTA Reporting System for Thyroid Cytology Useful for Risk-Classification of Thyroid Follicular Neoplasm?

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Background and Objectives: For follicular pattern lesions, thyroid fine-needle aspiration (FNA) cytology is considered a screening test, and a diagnostic lobectomy for histological diagnosis is recommended to patients with FN cytology in most international reporting systems. It means that more than 75% of patients found to have a benign lesion suffer from an unnecessary surgical treatment. The Japan Thyroid Association (JTA) published a new reporting system for thyroid cytology (JTA system). There are two points where the JTA system differs from existing internationally-accepted ones. The first is the sub-classification of so-called indeterminate category, which is divided into 'follicular neoplasm' and 'others'. The second is the sub-classification of follicular neoplasm (FN) into three subgroups based on cytological characteristics and probability of malignancy. One of the aims of the JTA system is effective triage of patients with higher risk of malignancy to surgery, and reduction of unnecessary surgery in patients with low-risk FN cytology. We herein report the usefulness of the JTA system.

Subjects and Methods: Between October 2013 and September 2014, US guidance FNA cytology was performed and followed by thyroidectomy in a total of 106 patients with FN cytology at Yamashita Thyroid and Parathyroid Clinic. These comprised 119 nodules that were histologically diagnosed postoperatively (102 benign and 17 malignant nodules). The first author classified all the nodules prospectively into 4 subgroups (benign, FN favor benign, FN borderline, FN favor malignant) based on the JTA system cytologically. The resection rate and risk of malignancy in the 4 subgroups were evaluated.

Results: One hundred two benign nodules included 74 adenomatous nodules, 26 follicular adenomas, and 2 lymphocytic thyroiditis, and 17 malignant nodules included 14 follicular carcinomas and 3 papillary carcinomas. The resection rates in 4 subgroups were 30.3, 53.5%, 87.5%, 100%, respectively. The risks of malignancy at histology in 4 subgroups were 7.1% (6/84 nodules), 20.0% (3/15 nodules), 33.3% (3/9 nodules), 45.5% (5/11 nodules), respectively.

Conclusion: We could successfully stratify patients with FN cytology according to the JTA system. The JTA system may enable us to select patients with follicular tumor for thyroidectomy to avoid unnecessary operations.

Disclosure of Interest: None declared.

P-168

Galectin-3 Expression on the Cellblocks from Fine Needle Aspiration of Thyroid Lesions

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Introduction: Fine Needle Aspiration Cytology (FNAC) with cellblocks is a good modality of choice for the thyroid-nodule evaluation. However, 15 to 30% of FNACs yield indeterminate cytological diagnosis. Galectin-3 has been reported to be a very sensitive and reliable diagnostic marker for preoperative identification of thyroid carcinomas with high sensitivity and specificity in cytological cell blocks. This marker was suggested as an indicator of malignancy and a potential tool in the differential diagnosis of the follicular patterned lesions of the thyroid. Galectin-3 polypeptide is a member of the oligosaccaride-selective binding protein family known as lectins. Galectin-3 plays important roles in cell-cell and cell-matrix interactions, extracellular matrix organization, cell growth and apoptosis, neoplastic transformation and metastasization. We performed Galectin-3 immunomarker on the cellblocks from FNA materials in thyroid lesions.

Aims and Objectives: To study the Galectin-3 expression on the cellblocks from FNA of thyroid lesions.

Materials and Methods: 100 patients diagnosed with thyroid lesions on FNA cytology were studied prospectively (2012–2014). Immunocytochemical expression of Galectin-3 was performed on cell blocks for each case. The pattern of expression for this marker (cytoplasmic or nuclear) in cells and its intensity were noted. Sensitivity and specificity were calculated taking histopathology as gold standard.

Results: Cellblocks from FNA from thyroid lesions were obtained from 100 patients. There were 15 males and 85 females in our study, age ranged from 17–70 years. In 62 aspirates, the cytological diagnosis was benign lesion (comprising of colloid nodule, hyperplastic nodule, Hashimoto's thyroiditis and adenoma). Galectin-3 expression in all these benign cases showed negative immunostainig (cytoplasmic or nuclear) except 2 cases (positive scoring and intensity as 1+ or 2+). In 36 out of 38 malignant cases, Galectin-3 expression was reported as strongly and highly positive (4+ or 3+) cytoplasmic or nuclear immunostaning as scoring or intensity in more than 10 percentage distribution in tumor cells. In 2 Out of 38 malignant cases, Galectin-3 epxpression was negative or showed poor staining (0–1+). The sensitivity and specificity of Galectin-3 on cellblocks as a marker for thyroid malignancies were 98.0% and 100% respectively. So Galectin-3 was found to be a highly sensitive marker for the diagnosis of thyroid neoplasms (papillary carcinoma and follicular variant of papillary carcinoma along with follicular carcinomas) in our study.

Conclusion: Galectin-3 immunocytochemistry was found to be a useful single marker on cell-blocks from fine needle aspiration in the differential diagnosis of thyroid lesions.

Disclosure of Interest: None declared.

P-169

Application of Thyroseq Study for Indeterminate Thyroid FNAs; Experience at a University Hospital

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Introduction: Thyroid cancer is the most common malignancy of the endocrine system in USA. FNA followed by cytological examination of thyroid lesions is a standard approach to detect thyroid cancer or to identify benign nodule. Results are reported as Bethesda Categories. Most thyroid malignancies can be diagnosed based on cytological features but in about 25% of thyroid nodules the presence of cancer cannot be ruled out by FNA cytology and is reported as indeterminate (Bethesda Categories III, IV, and V). Targeted mutation detection by next generation sequencing in thyroid FNA has been applied to detect cancer-related genes and gene fusions known to occur in thyroid cancer. Molecular studies or profiling for the Bethesda Categories III to V cases can be helpful in the clinical management of these patients. We report our preliminary experience with ThyroSeq V2, a targeted mutation detection system for thyroid specimens.

Methods: From January to September 2015 the Division of Cytology examined 159 Thyroid FNAs among which 87 cases were performed and interpreted by the senior author. Nineteen out of the 159 cases classified as Bethesda Categories III to V were submitted for ThyroSeq V2 tests and rate of mutation was calculated in each category. The senior author also performed 2nd review for all cases that had the mutation analysis done. Follow-up surgical resection, ultrasound examination and other relevant clinical outcomes were also collected.

Results: ThyroSeq V2 gene mutation analyses were performed in 12% of thyroid FNA cases. There were 14 cases of Bethesda Category III, 3 cases of Bethesda Category IV and 2 cases of Bethesda Category V. Two category V cases showed positive ThyroSeq results with one positive for HRAS mutation and the other positive for NRAS mutation. One category III was positive for NRAS and the other category III was positive for TSHR. No mutations was found in other 12 cases. In all 4 patients with positive mutation surgical histology confirmed thyroid cancer. In 2 cases with NRAS M+, one diagnosed with follicular carcinoma and the other one with papillary carcinoma. In 2 cases positive for HRAS M+ and TSHR M+, both had papillary carcinoma. The patients who had negative mutation and did have follow up for 3–6 months after FNA, showed no significant changes in the follow-up ultrasound.

Conclusions: Our preliminary results shows that ThyroSeq V2 next-generation sequencing could be a valuable complementary tool for accurate diagnosis of cancer in thyroid nodules reported as indeterminate in US/FNAs. Positive results have the potential to serve as a predictive indicator for thyroid cancer. Negative results in correlation with ultrasound features and 2nd review of cytology can be helpful for patient management.

Disclosure of Interest: None declared.

P-170

Exploiting Thyroid Cytomorphological Characteristics via Classification and Regression Trees

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Introduction: The aim of this study was to investigate the potential of classification and regression trees (CARTs) for cytological evaluation of thyroid lesions.

Methods: 521 histologically confirmed cases of thyroid FNAs prepared via liquid based cytology (LBC) were used. For each smear contextual and nuclear morphologic characteristics were described, using a systematic methodology. A Classification and Regression Tree (CART) model was trained on those data to predict the cytological result. Inadequate cases were excluded.

Results: The performance of the CART model using TBS III (AUS/FLUS) as cutoff point was Sensitivity = 89.64%, Specificity = 80.48%, PPV = 82.12, NPV = 88.60, FPR = 19.52%, FNR = 10.36% and Overall Accuracy = 85.06%; whereas if TBS IV (FN/SFN) was used as a cutoff point, the performance was 80.48%, 96.41%, 95.73%, 83.16%, 3.59%, 19.52%, 88.45% respectively. The ROC curve of the CART had AUC = 0.924, S.E.=0.013 Lower bound = 0.899, upper bound = 0.950 (C.I.=95%). Comparison of the two ROC curves for the Cytological diagnosis and the CART Model as these are produced against the histological diagnosis (being the golden standard), proved that there was no statistically significant difference between the two approaches (Difference = 0.011, Standard Error = 0.0231, z statistic = 0.476 and p = 0.6340).

Conclusions: Morphological evaluation of thyroid cytology characteristics via classification and regression trees had a performance comparable to standard cytological diagnosis. The approach has two major advantages: i) the creation of simplified rules that can be used during the everyday diagnostic procedure; ii) the potential to be deployed over the Internet infrastructure, thus enabling the non-experienced cytopathologist to introduce morphological characteristics in a web page; the outcome of this process is cytological characterization by the CART according to the Bethesda system and additionally an accompanying estimation of the probability of this 'diagnosis'. Applications include: decision support/tele-consultation, tele-education and homogenization of the diagnostic criteria.

Disclosure of Interest: None declared.

P-171

BRAF VE1 Immunocytochemistry on Cytospin Prepared Slides Using Residual Liquid-Based Cytology Materials from Papillary Thyroid Carcinoma Fine Needle Aspirations

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Objectives: BRAF V600E mutation is the most common genetic alteration in papillary thyroid carcinomas (PTCs) and has been utilized as a diagnostic and prognostic marker in PTCs. Recently, mutation specific antibody VE1 has been developed and shows a promising result in detecting BRAF V600E mutation in histologic preparation. However, the utility of this antibody in cytologic preparation is still under evaluation. The purpose of this study is to investigate the BRAF VE1 immunocytochemistry (ICC) on Cytospin prepared slides using residual liquid-based cytology (LBC) materials from PTC fine needle aspirations (FNAs) in comparison to the molecular testing result.

Materials and Methods: A total of 46 PTC cases diagnosed according to The Bethesda System for Reporting Thyroid Cytopathology were enrolled in this study. All the cases were histologically confirmed. The residual LBC materials were retrieved and an additional Cytospin prepared slide was obtained for BRAF VE1 ICC in each case. The results of BRAF molecular testing were obtained from the medical chart.

Results: Among the 46 cases, there were 26 (57%) positive for BRAF VE1 ICC using Cytospin prepared slides. The BRAF molecular testing results were available in 23 cases. Comparing with the molecular testing result, the BRAF VE1 ICC shows a sensitivity of 74% (14/19), a specificity of 75% (3/4), and a positive predictive value of 93% (14/15).

Conclusion: The BRAF VE1 ICC on Cytospin prepared slides using residual LBC materials can be successfully performed and revealed correlated results with the molecular testing. Based on the results, the BRAF status can be provided using this technique preoperatively along with the cytologic diagnosis.

Disclosure of Interest: None declared.

P-172

Impact of Noninvasive Encapsulated Follicular Variant of Papillary Thyroid Carcinoma on the Rate of Malignancy in Thyroid Nodules with a Preoperative Diagnostic Category of Follicular Neoplasm

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Objectives: Follicular variant of papillary thyroid carcinoma (FVPTC) is the most common type of malignant tumor among thyroid nodules with a preoperative diagnosis of follicular neoplasm (FN). Noninvasive FVPTCs have virtually no metastatic potential or risk of recurrence and are more akin to follicular adenomas. We aimed to determine the impact of noninvasive FVPTC on the rate of malignancy for preoperative FN diagnostic category.

Materials and Methods: From August 2012 to December 2014, 5,993 fine needle aspiration cytologies (FNAC) and 1,303 core needle biopsies (CNB) were performed to diagnose thyroid nodules at Seoul St. Mary's Hospital. We reviewed all patients who were preoperatively diagnosed with thyroid FN by FNAC or CNB and whose diagnosis was confirmed histologically after surgical resection.

Results: A total of 289 cases (117 FNAC and 172 CNB) were confirmed histologically after surgical resection. Of these, 131 (45.3%) cases were proven to be malignant in the resected specimens. After excluding noninvasive FVPTC from the malignancy group, the rate of malignancy for FN was 23.5% (68/131).

Conclusion: If noninvasive FVPTC were no longer considered malignancy, the rate of malignancy for preoperative diagnosis of FN would be significantly decreased. A change in terminology will affect the rate of malignancy of FN category.

Disclosure of Interest: None declared.

P-173

Cytologic Features of Adenoid Cystic Carcinoma-Like Pattern in Papillary Thyroid Carcinoma: A Case Report

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Objective: Adenoid cystic carcinoma-like pattern in papillary thyroid carcinoma is a rare phenomenon which may lead challenge in diagnosis. We present a case showing features of

adenoid cystic carcinoma-like pattern in fine needle aspiration (FNA).

Methods: FNA cytology material sent to our department for consultation. It was composed of smears stained with May Grünwald Giemsa and Papanicolaou stains.

Results: A 34-year-old male presented with a 2.5 cm nodule in the left thyroid lobe. FNA slides showed neoplastic follicular cells in microfollicular pattern and in few small sheets. The cells displayed oval enlarged nuclei with clear chromatin. Scattered intranuclear pseudoinclusions was also seen. Hyalen globules was detected in some of the microfollicules resembling adenoid cystic carcioma. The case was signed-out as papillary thyroid carcinoma consistent with follicular variant showing features suggestive of adenoid cystic carcinoma-like pattern. The patient underwent bilateral total thyroidectomy. Microscopic examination revealed papillary thyroid carcinoma with areas showing hyalen globules composed of basement membrane-like material. The tumor displayed both classical and follicular growth patterns without any features suggestive of rare variants of PTC such as columnar, cribriform/morular variant.

Conclusion: Adenoid cystic carcinoma-like pattern in PTC is a rare finding most frequently seen in follicular, columnar and cribriform/morular variants. Cytopathologists should be aware of these distinct features in thyroid tumours not to give an erroneous diagnosis of adenoid adenoid cystic carcinoma metastasis.

Disclosure of Interest: None declared.

P-174

A Case of Thyroid Papillary Carcinoma Forming a Mediastinal Mass with Marked Squamous Differentiation, Which Had Difficulty in Differentiation from Thymic and Lung Carcinomas

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Thyroid cancer rarely shows squamous cell carcinoma component. We hereby present a case of thyroid papillary carcinoma which formed a mediastinal mass with marked squamous differentiation and was difficult to be differentiated from thymic and lung cancers.

Case Presentation: A 48-year-old woman, who had presented left omalgia since 2 months before, consulted neighboring hospital. The computed tomography revealed an approximately 10 cm sized mass with cavitation in anterior mediastinum, a 2.5 cm sized ill-defined nodule of right thyroid and bilateral cervical lymph node enlargement. An aspiration biopsy cytology of the right cervical lymph node was performed and was diagnosed as metastatic thyroid papillary carcinoma. She was admitted to our hospital and underwent an aspiration biopsy cytology and a surgical biopsy of the mediastinal mass. The aspiration biopsy cytology showed numerous atypical squamous cells and only a small number of glandular atypical cells were also recognized. The surgical biopsy specimen was squamous cell carcinoma which was immunohistochemically positive for p40 and cytokeratin 5/6. Double carcinoma with thyroid papillary carcinoma and thymic or lung squamous cell carcinoma was suspected from the results of cytology and biopsy. Surgical resection of the mediastinal mass was performed after mediastinal radiation therapy (40 Gy) and anti-cancer chemotherapy (CDDP+VP16, 4 courses). The resected mediastinal mass was approximately 6.5 cm in diameter with cavities and partially involving peripheral region of the partially resected left lung. It histologically consisted of squamous cell carcinoma and papillotubular proliferation of atypical cuboidal cells. The squamous cell carcinoma component was focally positive for TTF-1. The papillotubular component was strongly positive for TTF-1 and thyroglobulin. Both components were negative for CD5 or c-kit which are characteristic for thymic carcinoma. The squamous cell carcinoma component showed markedly higher Ki67 labeling index than papillotubular component.

Discussion and Conclusion: The mediastinal mass of the present case was diagnosed as metastasis of thyroid papillary carcinoma with squamous differentiation because of the positive staining for TTF-1 and thyroglobulin. The small number of atypical glandular cells in the mediastinal aspiration cytology are considered to originate from the papillotubular component. In conclusion, some minor findings on cytology specimen could possibly play important roles for correct diagnosis.

Disclosure of Interest: None declared.

P-175

Papillary Carcinoma in Thyroglossal Duct Cyst Diagnosed by Ultrasound Guided Fine Needle Aspiration (FNA)

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Objectives: Neoplastic change in thyroglossal duct cyst is an uncommon event of less than 1%. Histological diagnosis is papillary carcinoma in 85% of cases. Reported sensitivity of preoperative FNA diagnosis is low due to the hypocellularity of the cyst fluid. Diagnostic accuracy could be improved if FNA is performed under Ultrasound guidance (UG).

Materials and Methods: In this case we discuss a case of papillary carcinoma of thyroglossal duct cyst diagnosed by UGFNA.

Result: A 39 year old woman was referred to Cytopathology lab for a USGFNA of a palpable mass in submental area of 6 months duration. Two passes with a 23G needle were done from the solid part of the lesion which was predominately cystic. Microscopically a typical papillary carcinoma of the thyroid characterized by the presence of papillary clusters and monolayered sheets of epithelial cells showing open nucleus with grooves and intranuclear cytoplasmic inclusions and areas of squamoid or microcystic cytoplasm, was seen. Microscopic diagnosis in correlation with the US and clinical findings was compatible with papillary carcinoma in

thyroglossal duct cyst. Appropriate management with Sistrunk's surgical procedure and total thyroidectomy was done.

Conclusion: Preoperative diagnosis of thyroglossal duct carcinoma is important since it alters patient management. Performing FNA under Ultrasound Guidance is essential for accurate cytologic diagnosis.

Disclosure of Interest: None declared.

P-176

Diagnosis of Pseudopapillary Variant of Medullary Thyroid Carcinoma by Fine-Needle Aspiration Cytology

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We report on a case of pseudopapillary variant of medullary carcinoma of the thyroid (MCT) in a 68 year old man who presented with bilateral thyroid nodules. The FNA sample showed small loosely cohesive clusters or single cells having eccentric, hyperchromatic round-oval nuclei with coarsely granular chromatin. In addition, amyloid deposits were observed in the background. MCT was strongly suspected morphologically, although a differential diagnosis with papillary carcinoma had to be performed, because branching vascular vessels were observed in close relation to the neoplastic cells. Based on positive immunocytochemical staining of the neoplastic cells for Calcitonin and CEA, a cytologic diagnosis of MCT was performed. In the resection tissue, the bilateral tumor showed papillary proliferations having hyaline vascular tumor stroma, and contained solid foci characteristic of typical MCT in some areas. The hyaline vascular tumor stroma was positive for Congo red stain and showed green birefringence under polarized light. In addition, the tumor cells strongly stained with calcitonin, chromogranin A and CEA and the neoplasm was diagnosed as MCT in its pseudo-papillary variant. In consideration of the presence in this variant of branching vessels showing adherence to the neoplastic cells, strongly simulating papillary carcinoma, the existence of a pseudo-papillary variant of MTC should be kept in mind and appropriate cytochemistry for Congo Red and immunocytochemistry for calcitonin should be performed for an accurate cytologic diagnosis.

Disclosure of Interest: None declared.

P-177

Primary Mucoepidermoid Carcinoma of the Thyroid Gland

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Introduction: Mucoepidermoid carcinoma is a rare primary thyroid tumor with indolent biologic potential. Two types of tumors have been described under this category: mucoepidermoid carcinoma (MEC) and sclerosing mucoepidermoid carcinoma with eosinophilia (SMECE). The MEC shows both squamous and glandular differentiation in a background of a noninflamed gland, whereas SMECE is characterized by extensive sclerosis, squamous and glandular differentiation, a concomitant inflammatory infiltrate rich in eosinophils, and a background of lymphocytic thyroiditis. The MEC occurs anywhere in the thyroid gland and appears as a partly circumscribed lesion, generally soft in consistency. It is most often found in female patients. The age of the patients range from 10 to 66 years.

Case Report: A 70-year old female undergone thyroid ultrasound because of routinely found elevated TSH levels. Thyroid scan showed multiple hypoechogenic nodules with hyperechogenic echoes in the right thyroid lobe, with multiple cervical lymph nodes on the same side of the neck. FNA of the above mentioned nodules revealed cellular smears, with partly papillary groups of atypical cells, some with intranuclear inclusions, some multinuclear giant cells and histiocytes. The finding was consistent with moderately differentiated thyroid papillary carcinoma. Total thyreoidectomy with radical neck dissection was performed. Histopathological diagnosis was mucoepidermoid carcinoma of the thyroid.

Conclusion: In the diagnosis of papillary thyroid carcinoma that is not well differentiated, we have to take in consideration the possibility of the existence of its subtypes, for example mucoepidermoid carcinom.

Disclosure of Interest: None declared.

P-178

A Case Report of Carcinoma Showing Thymus-Like Differentiation of Thyroid

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Objectives: Carcinoma showing thymus-like differentiation (CASTLE) is a rare carcinoma of the thyroid or adjacent soft tissue of the neck morphologically and immunohistologically similar to a thymic carcinoma. It was first named 'CASTLE' by Chan and Rosai in 1991, and only a few cases have been reported. Its incidence in thyroid malignant tumor is estimated at 0.075%. Thyroid CASTLE is now recognized as an independent clinicopathological

entity and is included in the World Health Organization's classification of tumors of endocrine organs.

Materials and Methods: We report a case of CASTLE diagnosed by thyroid aspiration. This 52 years old woman complaint that she has enlarged anterior neck mass recently. It was mild tenderness. There was no body weight loss. No syndrome and signs related hyper or hypothyroidism. She also has hoarseness for more than 20 years. Computerized tomography (CT) revealed a 4.8 cm x 3.5 cm soft tissue mass over right anterior thyroid region. Thyroid ultrasonography showed a hypoechoic mass measuring 4.1 × 3.3 × 3.6 cm that had replaced the majority of the right thyroid gland. The mass had a smooth margin. Fine-needle aspiration (FNA) of right thyroid nodule was performed, and standard Papanicolaou and Liu's stains were performed on alcohol-fixed and air-dried direct smears, respectively.

Result: The smear was composed of cohesive three dimensional clusters and sheets or singly scattered cells. In some foci, there was nuclear overlap and a suggestion of nuclear molding. Many stripped nuclei were present in the background. The tumor cells showed mild anisonucleosis and high nuclear: cytoplasmic (N/C) ratios. Nuclei were round to ovoid with irregular nuclear contours and small, distinct nucleoli. The nuclear chromatin was vesicular or coarsely granular. Cytoplasm was scanty, and cytoplasmic border was indistinct. She received bilateral total thyroidectomy after cytology report. The sections reveal a lobulated neoplasm composed of syncytial sheets and nests of tumor cells with indistinct cell borders, moderate cytoplasm and vesicular nuclei separated by fibrous septae containing focal lymphoplasmacytic infiltrate. Occasional tumor islands showed central foci of squamoid differentiation and neuroendocrine differentiation. The immunohistochemical stains of tumor cells are TTF-1(–), thyroglobulin(–), CD56(–), synaptophysin: focal weak(+), CK(+), p63(+), CD5(+) and Bcl-2(+), Chromogrnin-A focal weak(+).

Conclusion: Although CASTLE is rare, it should be distinguished from poorly differentiated tumors of the thyroid region and the use of ancillary studies are essential to diagnose this rare entity associated with a relatively favorable prognosis.

Disclosure of Interest: None declared.

P-179

The Liquid-Based Cytology of a Carcinoma Showing Thymus-Like Differentiation in the Thyroid (CASTLE)

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Objectives: A case of carcinoma showing thymus-like differentiation in the thyroid (CASTLE) was reported.

Materials and Methods: The clinicopathologic features, liquid-based cytology slides from preoperative fine needle aspiration, intraoperative imprint cytology in Liu's stain, Hematoxylin and eosin (H&E) stains, and immunohistochemical stains of the tumor were reviewed.

Results: A 38 year-old lady complained of persistent hoarseness for 3 months with intermittent chocking. The laryngoscope showed incomplete glottic closure with right vocal paralysis. The neck ultrasonography revealed a right thyroid mass. The liquid-based cytology from fine needle aspiration showed hypercellularity and cohesive three dimensional sheets of atypical cells which revealed high nuclear: cytoplasmic (N/C) ratios, round to ovoid nuclei, irregular nuclear contours, and distinct nucleoli with moderate degree of lymphocytes infiltrate in the background. The nuclear chromatin was coarsely granular. Focal squamoid keratinization is found. Nuclear grooves and intranuclear cytoplasmic inclusions were not observed. Cytoplasm was scanty and amphophilic with an indistinct border. Mitotic figures were rarely seen. Papillary or follicular structure was not observed. The aspirate was interpreted as suspicious of malignant neoplasm without further definitive classification. Computerized tomography (CT scan) revealed a right thyroid tumor with compression to trachea. With intraoperative consultation, she received radical thyroidectomy and central neck lymph node dissection. Grossly, in the right thyroid, there was an ill-defined gray white infiltrating tumor, measuring 2.7 x 2.5 x 1.6 cm. Microscopically, the tumor consisted of lobules of solid sheets of epithelial cells with focal whorls resembling Hassall's corpuscles, variable lymphocytes infiltrate, and separated by fibrous bands. Tumor cells had indistinct cell borders, ill-defined cytoplasm with eosinophilic or amphophilic cytoplasm, round to oval vesicular nuclei, and prominent nucleoli. Immunohistochemically, tumor cells were positive for cytokeratin, p63, p40, CD5, and CK5, but negative for thyroglobulin, TTF-1, CD56, and TdT. Extensive extrathyroid tumor invasion into muscles and perithyroid soft tissues were also present with one metastatic lymph node. She received postoperative radiotherapy for tumor involvement in resection margins, and so far got no evidence of tumor recurrence for three months.

Conclusion: CASTLE is rare and, among the reported cases, the discussion on its cytologic findings, especially liquid-based cytology, is extremely limited. We presented a typical case for the cytomorphological correlation between cytology and histology.

Disclosure of Interest: None declared.

P-180

Is Liquid Based Cytology (LBC) Better Than Conventional Smears Made from FNA of Thyroid Performed by the Cytopathologists? A Split-Sample Comparative Analysis

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Objective: Fine-needle aspiration cytology is the best tool for initial evaluation of thyroid lesions. In our institution, FNA is performed by the cytopathologists and routinely conventional smears are prepared and stained by MGG and H&E stains. Hence the objective of this study was to compare cytoptahologist prepared conventional smears (CS) versus LBC (SurePath, BD) preparations in thyroid FNA samples for diagnostic efficacy. Secondary objective was to evaluate the differences in cytomorphological features.

Samples and Method: Prospective randomized study by split-sample method over 1½ years. The cytodiagnosis was as per the Bethesda system for reporting thyroid cytopathology (TBSRTC). Cyto-morphological features including cellularity, cell types and background were evaluated in conventional smears (CS) and corresponding LBC preparations. Finally they were categorized into 3 groups-(1) CS and LBC equivalent for diagnosis (2) CS better than LBC (3) LBC better than CS for providing the diagnosis.

Results: A total of 200 non-consecutive thyroid FNA cases were evaluated. Overall in 59% cases, LBC was equivalent to conventional smears; in 37% cases CS was better than LBC for providing the diagnosis. LBC did not improve the unsatisfactory rate. LBC was better than CS in only 5 cases, which included 4 cases of colloid goiter and one case of follicular neoplasm. The differences were analyzed in each TBSRTC category. In malignant lesions LBC was not better than CS in any case. In category 4, LBC was superior to CS in just 5% cases; the two were equivalent in majority of cases. Microfollicles and nuclear features of Papillary carcinoma thyroid were better appreciated in CS.

Conclusion: In thyroid FNAB cases, cytopathologists prepared conventional smears are superior to LBC preparation in terms of cellularity and diagnostic efficacy across all categories of TBSRTC. Hence, LBC preparation is merely complementary and should not replace conventional smear techniques.

Disclosure of Interest: None declared.

P-181

The Fine Needle Aspiration Cytology of Primary Thyroid Lymphoma: A Case Report

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Objectives: A demonstration of the cytological features of primary thyroid large B-cell lymphoma.

Materials and Methods: The clinical presentations, conventional cytology from preoperative fine needle asipration, and histological correlation of the tumor after surgery are reviewed.

Results: This is a 74 year-old man who had hypertension for years. He suffered from a rapid growth mass over right lateral neck for one month. A 6 cm non-tendered and movable mass was noted over right neck (level III region) through physical examination. No lesion was found during nasopharyngoscopic examination. CT scan showed a 4 x 2.9 x 6.3 cm low density lesion over the right lobe of thyroid gland. No lymphadenopathy was found. All the related lab data included serum TSH and free T4 were within normal limit, except thyroglobulin were high (88.25 ng/ml). Fine needle aspiration cytology exam through conventional method was performed later. The cytological features showed many scattered small-sized lymphocytes and large-sized lymphoid cells with prominent nucleoli. Many apoptic bodies were seen in the background. The aspirate was interpreted as atypical lymphoid cells suggested further survey. Under the impression of thyroidal tumor, right thyroid lobectomy was performed. Grossly, the tumor, measuring 7 x 5 x 3.3 cm and 61.6 gm in weight, occupied most of the thyroid. The tumor cut surface was smooth, pale white-grey with a 'fish-flesh' appearance. Microscopically, the tumor composed of diffuse proliferation of large cells predominantly. Some nodular aggregates of neoplastic lymphoid cells were seen. There were many apoptotic bodies identified. The residual thyroid tissue showed lymphocytic infiltration in places. The neoplastic lymphoid cells were immunoreactive for CD20 and CD10, while negative for CD3 and bcl-2, immunohistochemically. According to the histological and immunohistochemical findings, diffuse large B-cell lymphoma, germinal center-like subgroup was most likely. The patient had regular follow-up without tumor recurrence after surgery for two months so far.

Conclusions: Primary thyroid lymphoma is uncommon, and discussion focused on cytological and histological correlation is limited. Therefore, we present a case of primary large B-cell lymphoma of thyroid with cytohistological correlation.

Disclosure of Interest: None declared.

P-182

Thyroid Extramedullary Hematopoiesis Diagnosed by Fine-Needle Aspiration Cytology

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Objectives: A case of extramedullary hematopoiesis (EMH) in the thyroid was reported.

Materials and Methods: The clinicopathologic features, cytology slides from fine needle aspiration in Liu's stain and Papanicolaou stain were reviewed.

Results: A 57 year-old Taiwanese woman complained of right neck lump noted for one month. She visited the endocrinologist for help. On physical examination, there was a vague mass-like lesion over right side of anterior neck without tenderness. Thyroid function tests showed normal serum T3 and T4 levels. Neck ultrasonography revealed an ill-defined mass-like lesion in the right thyroid, in suspicious of nodular goiter. Herein, fine needle aspiration was performed. Cytologically, both the wet and dry smears were hypercellular and revealed trilineage hematopoiesis and typical megakaryocytes with multilobated hyperchromatic nuclei and abundant cytoplasm admixed with orthochromic and polychromatophilic normoblasts, and myeloid precursors at various stages of maturation. EMH was diagnosed by fine-needle aspiration cytology. However, the patient was lost to follow-up, and histological correlation and detail clinical information were not rendered. EMH is extremely rare and usually asymptomatic, but it could produce organomegaly or tumor-like masses involving any organ. Thyroid is one of the rarest sites of EMH, while more than 95% of cases involve the liver, spleen, and lymph nodes, as these organs are primarily responsible for hematopoiesis in the fetus. The cytologic differential diagnosis might include granulocytic sarcoma, inflammatory pseudotumor, lymphoma, inflammatory disorders, and other lesions containing multinucleated giant cells. Most cases could be diagnosed based on cytomorphologic features alone, especially when trilineage hematopoiesis is recognized.

Conclusion: We present a rare case of EMH in the thyroid diagnosed by fine-needle aspiration cytology with incomplete clinical surveillance. We aim to enhance awareness of this interesting yet rare entity.

Disclosure of Interest: None declared.

P-183

Cancelled

P-184

Pre-Surgical Differentiation of Parathyroidal Tumors by Fine-Needle Cytology: Is It Reliable?

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Background: Clinical hyperpatyroydism is determined by parathyroid adenoma (PA) or carcinoma (PC) in much of the cases. Corresponding lesions require surgical treatment but its extension (nodulectomy vs. lobectomy) may differ accordingly. Fine needle cytology (FNC) diagnosis of parathyroid lesions may be performed and confirmed by the parathyroid hormone detection by immunocytochemical positivity on additional smears or by immunoassay on needle washing. Nonetheless the differentiation between PA, atypical PA and PC is considered very difficult or impossible on FNC. Therefore corresponding tumours are generally diagnosed as 'parathyroid neoplasm' (PN) on FNC samples. We retrospectively revised a series of PN-FNC, histologically controlled, with the aim of identify cytological criteria that might be useful to further classify FNC-PN.

Materials and Methods: Thirty consecutive FNC of PN, collected over 6 years were reviewed by two of the Authors (AC, PZ). Evaluated cytological criteria were: low or high cellularity, presence of tightly or loose cohesive groups, acinar or solid pattern, small or large nuclear size, presence or absence of nuclear atypia, dense or granular chromatin pattern, presence or absence of nucleoli. All the cases were blindly evaluated in a binary score system by two of authors (AC, PZ), subsequently merged in a single classification and compared to the corresponding histological controls.

Results: All the PA, including two cases histologically diagnosed as atypical PA, showed quite repetitive and homogeneous patterns represented by tightly cohesive cell groups, microacinar pattern, small nuclei, dense chromatin pattern and absence of nucleoli. Conversely two PC showed high cellularity, loose cohesive cell groups, solid pattern, large nuclear size, nuclear atypia, granular chromatin pattern and presence of nucleoli.

Conclusions: FNC features of parathyroid neoplasm, confirmed by parathyroid hormone detection, may suggest the presurgical differentiation between PA and PC being atypical PA indistinguishable from the ordinary ones. Further observations are needed to confirm this preliminary study.

Disclosure of Interest: None declared.

Liver and Pancreas / Gastrointestial

P-185

Cytological Diagnosis and MUC Stainings of Intraductal Papillary Mucinous Neoplasms Using Cytological Specimens of Pancreatic Juice

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Objectives: Intraductal papillary mucinous neoplasms (IPMN) show noninvasive expansive growth and a favorable outcome. IPMN were classified into low- or intermediate-grade dysplasia (IPMN-LGD), and high-grade dysplasia (IPMN-HGD) and IPMN with an associated invasive carcinoma. As some IPMN progress into invasive ductal carcinoma (IDC), it is very important to assess the malignant potential of IPMN to decide surgical indication. In this study, we investigated the morphological features and the expression profiles of mucins of cytological specimens of pancreatic juice using the cell transfer method.

Materials and Methods: Between October 2008 and December 2015, 19 patients who were referred to our hospital for a diagnosis of IPMN were included in this study. Nineteen patients underwent ERCP for assess the dilated pancreatic duct and collected pancreatic juice samples. The pancreatic juice was mixed with 10 ml of Hanks solution (Nissui, Tokyo, Japan) at 4°C immediately and transferred to the laboratory.

Cytological Diagnosis and MUC Staining: The medium was centrifuged again for 1 min, and the cell pellet was smeared onto 2 glass slides and fixed in 95% ethanol. The specimens were stained with Papanicolaou method, and cytological diagnoses were made. After the morphological diagnosis, the same cytological specimens underwent staining for MUC 1, 2, 5AC, and 6 using the cell transfer method (Malinol, Muto chemical, Japan). The final diagnosis was made based on surgical specimens.

Results: Using Papanicolaou staining, 11 cases were diagnosed as IPMN-LGD and 8 cases were diagnosed as IPMN-HGD or IDC. The expression profiles of these cytological specimens showed that 10 cases were classified as gastric subtype, 2 cases as intestinal subtype, and 7 cases as pancreatobiliary subtypes. The final diagnosis revealed that 8 cases were IDC, 2 cases were IPMN with an associated invasive carcinoma, 6 were IPM-HGD, and 9 were IPMN-LGD. In 15 patients, the mucin expression profiles in the cytological specimens agreed with the profile in the surgically resected specimens. Two patients out of 11 cases of IPMN-LGD were positive MUC1 staining in cytological specimens, and revealed IPMN with an associated invasive carcinoma in surgically resected specimens. Using cytological features and MUC stain, the diagnosis of IPMN-HGD/IDC showed 72.7% sensitivity, 75% specificity, and 73.7% accuracy.

Conclusion: The histological subtyping using pancreatic juice with MUC staining is clinically useful.

Disclosure of Interest: None declared.

P-186

Use of LBC in EUS-FNA of Pancreatic Lesions

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Objectives: Endoscopic ultrasound-guided Fine-Needle Aspiration (EUS-FNA) is carried out for the diagnosis of pancreatic lesions (PL), but its repetition is difficult for patients because of its invasiveness. Although rapid on-site cytological evaluation (ROSE) is recommended, it is difficult in practice because it is labor intensive. Therefore, we focus on liquid-based cytology (LBC) specimens. LBC allows the use of all collected samples and is a routine method of preparing specimens. In cervical cytology, LBC has been shown as contributing to reduction of inadequate specimens, and being a practical method of preparing specimens of high diagnostic potency. Here, we describe the diagnostic efficacy and use of the LBC technique without ROSE.

Materials and Methods: The subjects, 157 patients with suspected pancreatic carcinoma (PC), were examined by EUS-FNA at Nara Medical University Hospital between 2011 and 2014. The PC was finally confirmed with the use of surgically resected specimens and clinical follow-up. The number of needle passes (NP) was mainly two, but aspiration was repeated when samples were difficult to obtain macroscopically. Samples were collected in Cytorich Red (CR; BD Diagnostics, Franklin, NJ, USA): un-shredded samples were transferred into 10% neutral buffered formalin, and shredded ones were left in CR. The procedure for preparing LBC specimens followed the manual protocol recommended by BD. We examined the yield of specimens by cytological analysis (YSCA) and the diagnostic performance, which included sensitivity, specificity and accuracy. Moreover, YSCA and its accuracy were assessed by 62 consecutive aspirations on 28 patients between January and July of 2013. Additionally, the remaining LBC samples were subjected to immunohistochemical staining (IHCS) for the definitive diagnosis.

Results: Final diagnoses of PL confirmed 126 cases of PC, 14 of autoimmune pancreatitis, 3 of tumor-forming pancreatitis, 3 of primitive neuroectodermal tumor (PNET) and 11 other types. The mean number of NPs was 2.2 (range: 1–4), the YSCA rate 100%, sensitivity 93.7%, specificity 96.8% and accuracy 94.3%. Moreover, at each aspiration, the YSCA rate was 90.3% and the accuracy 82.1%. Also, IHCS of synaptophysin (clone 27G12; Nichirei, Japan) against 2 samples suspected with PNET was positive.

Conclusion: Although tissues collected by EUS-FNA are microscopic, the LBC technique provides good diagnostic efficacy even with a small number of aspirations and is highly efficient for collecting cells, thus contributing to saving time and labor, standardizing sample treatment methods, and allowing IHCS of remaining samples. Therefore, we consider that the LBC technique improves diagnostic accuracy when ROSE is not conducted. 0744-22-3051.

Disclosure of Interest: None declared.

P-187

Endoscopic Ultrasound-Guided FNA in the Diagnosis of Solid Pancreatic Lesions

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Objective: Endoscopic ultrasound is used in the diagnostic evaluation of suspect lesions of pancreas and in tumour staging. In addition, EUS-FNA can supply a morphological diagnosis in patients chosen for oncological treatment. In the neoadjuvant treatment setting, it is important to know the accuracy of EUS-FNA of pancreatic tumors. The procedure is done under light sedation/analgesia. A cytopathologist or cytotechnician present 'on-site' is important for the end diagnostic result. The aim of this study was to evaluate the accuracy of EUS-FNA of solid lesions of pancreas.

Methods: 97 FNAs from 91 patients with solid pancreatic tumors were identified in the pathology journal of Oslo University Hospital Ullevål from 2010–2014. Diagnostic accuracy was evaluated with regard to ability to distinguish benign and malignant lesion. Cytology was checked with histology, radiology and/or clinical follow-up for minimum 3 months.

Results: 5 minor, and no major, complications were registered. Adequacy of cell material was 98%. Diagnostic accuracy was 90%, sensitivity 91%, specificity 86%, negative predictive value 94% and positive predictive value 81%.

Conclusions: A close cooperation between clinician and pathologist, as well as use of ancillary studies, contribute to a high accuracy of EUS FNA of solid pancreatic lesions. The following challenges were noted: 1. To aspirate diagnostic material from some ductal adenocarcinomas due to tumor desmoplasia and few atypical cells; 2. discrete cytological atypia in tumors with high differentiation can be difficult to distinguish from reactive changes in pancreatitis; and 3. The existence of many rare tumor types in pancreas. Pathologist on site improves the diagnostic outcome. The procedure is safe with few complications.

Disclosure of Interest: None declared.

P-188

Metastatic Neoplasms to the Pancreas Diagnosed by Fine Needle Aspiration/Biopsy Cytology: A 15-Year Retrospective Analysis

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Introduction: With the advent of endoscopic ultrasound (EUS) guidance, fine needle aspiration (FNA) and core needle biopsy (CNB) have been increasingly utilized in the evaluation of pancreatic masses. Despite this, metastatic tumors to the pancreas are only rarely encountered. In our study we aspired to determine the incidence and primary origin of all metastases to the pancreas at our institution, to examine their clinicopathologic and cytomorphologic features, and to ascertain the effect of EUS guidance implementation.

Methods: A Natural Language Search of our CoPath database was undertaken to review all pancreatic FNAs and/or CNB examined from January 2000-December 2014.

Results: During our 15-year study, 636 patients underwent pancreatic FNA/CNB including 252 computerized tomography (CT) guided and 384 EUS-guided biopsies. 221 (35%) were malignancies. 16 were metastases to the pancreas, comprising 2.5% of biopsies and 7.2% of malignancies. Rapid on-site evaluation (ROSE) was used in 14 (88%). 11 (69%) patients were male and 5 (31%) female, aged 43–82 years. 15 (94%) were solitary lesions. The metastases were in the head (7.44%), body (5.31%), and tail (4.25%), with size ranging from 1.2–10.9 cm (mean 4.2 cm). The most common primary site of origin was lung (6.38%), including 3 adenocarcinomas, 2 small cell and 1 adenosquamous carcinoma. There were 3 (19%) each of renal and gastrointestinal tract malignancies, including 3 clear cell renal carcinomas, 2 mucinous colonic and 1 gastric adenocarcinomas. The remaining cases included malignant melanoma, Merkel cell carcinoma, gallbladder small cell carcinoma, and olfactory neuroblastoma. Confirmatory immunohistochemistry was employed in 11 (69%). There was a prior history of malignancy in 14 (88%). The time from primary cancer diagnosis to pancreatic metastasis detection varied greatly (1 week-11 years). The majority (81%) were deceased within 2 years of pancreatic metastasis. EUS guidance was implemented in 2008. Metastases comprised 3.2% and 2.1% of pancreatic CT and EUS-guided biopsies, respectively. Therefore, there was no difference in the detection rates of pancreatic metastases between CT and EUS-guided biopsies. Furthermore, there was no difference in metastasis size, tumor distribution, or ROSE utilization between the 2 diagnostic modalities.

Conclusion: Metastases to the pancreas diagnosed by FNA/CNB are rare in our institution, comprising only 2.5% and 7.2% of total and malignant pancreatic FNA/CNB, respectively. FNA/CNB proved to be an effective modality in the diagnosis of metastases to the pancreas, thereby obviating the need for further invasive procedures. We also present the 1st known case of a metastatic olfactory neuroblastoma to the pancreas diagnosed by needle biopsy cytology.

Disclosure of Interest: None declared.

P-189

Cytology Assessment Can Predict Survival for Patients with Metastatic Pancreatic Neuroendocrine Neoplasms

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Objectives: Pancreatic neuroendocrine tumors (PanNETs) commonly metastasize to the liver and the Ki67 index (KI) is useful to predict prognosis and guide therapy. There is limited knowledge of the predictive value of KI on cytology in this context. Fine

needle aspiration (FNA) may offer advantages for KI assessment because the technique obtains highly cellular, well-preserved specimens with potential for broader tumor sampling. We evaluated KI and cytomorphology for correlation with survival.

Materials and Methods: Patients with metastatic PanNET were identified from a prospectively maintained hospital database (2001–2013). Cytomorphology of liver FNAs was reviewed and Ki67 immunostain was applied to concurrently obtained cell block (CB), core biopsy (BX), and/or de-stained cytology smear/Thinprep (SM). KI was assessed by a manual count of hotspots and assigned WHO grade G1-3. Clinical follow up was available for all patients. We evaluated association of overall survival [OS] with an aggregate cytology grade (CG) (defined as the SM grade or CB if no SM) by using Cox regression adjusting for tumor stage. Secondly, we evaluated the association of cytomorphology with OS using unadjusted Cox regression.

Results: The cohort comprised 40 patients of median age at diagnosis 61.5 years with 83% presenting stage IV. The distribution of morphologic differentiation was 34 (85%) well (WD) and 6 (15%) poor (PD). Mean KI for WD was 13% (0–42%) and PD was 47% (30–60%). Agreement for differentiation between SM and CB was 29/33 (88%); SM and BX was 12/13 (69%). [t2] Agreement for grade between SM and CB was 19/29 (66%); SM and BX was 7/16 (44%). The distribution of the aggregate cytology grade was: G1 = 4; G2 = 26; G3 = 10 (WD = 5 and PD = 5) which strongly predicted survival with a hazard ratio [HR] of 1.9 (95% CI: 1.2–2.9) for each ascending grade. Median survival projections (months) by CG were G1 = 121 (95% CI: 57–185 (estimated)); G2 = 45 (95% CI 29–87); and G3 = 19 (95% CI 1–44). For WD (n = 34), grade and KI were not significant predictors of OS (all P>.1). Certain cytomorphologic features had a strong association with survival in unadjusted analysis: differentiation (median survival (months) for WD = 45; PD = 3; (P < 0.001)), necrosis (P = 0.007), conspicuous mitoses (P = 0.007), and apoptosis (P < 0.001); oncocytic features and nuclear size were not associated with survival differences.

Conclusions: Pan-NET grading on cytology may not exactly correlate with concurrent core biopsy, but morphological differentiation and KI are good predictors of survival based on stage-adjusted analysis.

Disclosure of Interest: None declared.

P-190

Pancreatic Neuroendocrine Tumors Diagnosed by Fine Needle Aspiration/Biopsy Cytology: A 15-Year Retrospective Analysis

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Objectives: With the advent of endoscopic ultrasound (EUS) guidance, fine needle aspiration (FNA) and core needle biopsy (CNB) have been increasingly utilized in the evaluation of pancreatic masses. With EUS, some studies have reported an increased detection of pancreatic neuroendocrine tumors (PNET). In our study, we aspired to determine the incidence of PNET at our institution, to examine their clinicopathologic and cytomorphologic features, and to ascertain the effect of EUS guidance implementation.

Materials and Methods: A Natural Language Search of our CoPath database was undertaken to review all pancreatic FNAs and/or CNBs examined from January 2000-December 2014. Statistical analysis was performed with Fisher's exact and student t-tests.

Results: During our 15 year study period, 636 patients underwent FNAs and/or CNBs of the pancreas, including 252 computerized tomography (CT) guided and 384 EUS-guided biopsies. 221 (35%) were malignancies. Thirty-five were PNETs, comprising 5.5% of all needle biopsies and 15.8% of malignancies. Twenty-one (60%) patients were male and 14 (40%) female, ranging in age from 45–89 (mean 64). Thirty (86%) presented with a single nodule. Twenty-one (60%) were in the tail/body, 10 (29%) in the head/neck/uncinate, and 4 (11%) were multifocal. Tumor size varied from 0.7–10 cm (mean, 4.0 cm; median, 3.2 cm).

EUS guidance was implemented in 2008. PNET comprised 3.2% and 7.0% of pancreatic CT-guided and EUS-guided biopsies, respectively (P < 0.05). EUS was able to detect significantly smaller tumors (range 0.7–9 cm, mean 3.4 cm) than did CT (range 1.5–10 cm, mean 5.7 cm) (P < 0.05). There was no significant difference in tumor localization in the pancreas or ROSE utilization between the 2 diagnostic modalities.

Six (17%) FNA/CNB were not diagnostic for PNET, including 2 CT and 4 EUS-guided cases. One of these nondiagnostic cases was the smallest (0.7 cm) tumor in our study. However, size was not a factor in the remaining cases (ranges 0.7–10 cm). There was a single false positive FNA/CNB case which proved to be a benign congenital cyst upon pancreatectomy.

Rapid on-site evaluation (ROSE) was used in 32 (91%) cases. The ROSE diagnosis correlated with the FNA/CNB biopsy in all cases except one. Upon review, the PNET was misdiagnosed as lymphocytes on ROSE, proving to be a pathologist interpretation error.

Conclusion: The sensitivity and specificity of pancreatic FNA/CNB for detecting PNETs were 97% and 99%, respectively. EUS-guidance improved the detection of smaller PNETs, resulting in a statistically significant increase in PNET diagnoses at our institution.

Disclosure of Interest: None declared.

P-191

Primary Pancreatic Lymphoma: A Case Report and Literature Review

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Objectives: To present a case report of a primary pancreatic lymphoma of a 61 year old male diagnosed on fine needle aspiration (FNA) cytology.

Materials and Methods: The investigation and diagnosis of this case was managed using endoscopic ultrasound with fine nee-

dle aspiration, cytology and ancillary techniques (cell block, immunohistochemistry), and flow cytometry.

Results: A 61 year old male was referred to theRoyalFreeHospital, London, for investigation of a pancreatic mass. The endoscopic ultrasound showed a 6.7 cm hypoechoic and homogenous mass and material was taken for cytology. The cytological preparation showed large atypical lymphoid cells in keeping with a lymphoma. Material was sent for a cell block and immunohistochemistry which showed B cell clonality. The case was reviewed by a specialist haematopathologist who diagnosed diffuse large B cell lymphoma.

Conclusion: Primary pancreatic lymphomas account for less than 3% of pancreatic neoplasms. If suspicion is raised on abnormal radiology and appropriate samples are taken, then a combination of cytology, immunohistochemistry and flow cytometry can provide a diagnosis, preventing unnecessary surgery and ensuring the patient is treated correctly.

Disclosure of Interest: None declared.

P-192

An Abnormal p53 Immunohistochemical Result in 'Atypical' Pancreatobiliary Brushings Is Highly Correlated with a Diagnosis of Malignancy

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Objectives: Pancreatobiliary brush cytology is frequently performed in patients with strictures involving the pancreaticobiliary tree. Reported sensitivities and specificities have ranged from 30–60% and 87–100%, respectively. Pancreatitis, lithiasis, primary sclerosing cholangitis and prior stenting make interpretation challenging. In our institution we report cytology findings as nondiagnostic, benign, atypical favor reactive, atypical, atypical suspicious for malignancy and malignant. Inexpensive, readily available ancillary studies to increase the diagnostic accuracy of biliary brushings are needed. In surgical pathology material, combined use of p53 and MUC4 immunostains has been shown to be useful in distinguishing pancreatic adenocarcinoma from chronic pancreatitis. Focusing our study on cases previously classified as 'atypical,' we hypothesized that these markers would be similarly useful in cell block material from pancreatobiliary brushings.

Material and Methods: 28 pancreaticobiliary brushings diagnosed as atypical favor reactive (10 cases), atypical (9 cases) and atypical suspicious for malignancy (9 cases) with available cell block material were retrieved from our Cytology files. Cases were originally diagnosed between January 2010 and September 2015. The 'gold standard diagnosis' was based on surgical and/or clinical follow up, which was obtained for all patients. Immunohistochemistry (IHC) for p53 and MUC4 was performed on cell blocks. p53 was interpreted as normal/wild-type (weakly staining) or abnormal/mutant (diffuse, strong staining); MUC4 was scored as positive (more than 5% cells staining) or negative. IHC results were compared to the gold standard diagnosis.

Result: Among 13 cases of clinically confirmed carcinoma, p53 was mutant pattern in 8 (62%), while MUC4 was positive in 10 (77%). In 15 clinically benign cases, p53 was mutant pattern in 1 (6%), while MUC4 was positive in 13 (87%). Thus, p53 was 62% sensitive and 93% specific, and MUC4 was 77% sensitive and 13% specific for carcinoma. Specificity and sensitivity for each category (atypical favor reactive, atypical and atypical suspicious for malignancy) were 100% and 100%, 66% and 66%, and 57% and 100% respectively.

Conclusion: Although only moderately sensitive, an abnormal p53 result on cell block material from pancreatobiliary brushings considered 'atypical' based on cytomorphologic criteria is highly predictive of malignancy. MUC4 was not useful in this diagnostic application.

Disclosure of Interest: None declared.

P-193

Cytological Differentiation of Well-Differentiated Adenocarcinoma and Benign Atypical Cells Using Bile Cytology Quantification

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Objectives: We report our attempt to quantify cytological findings to select significant findings in the differentiation of well-differentiated adenocarcinoma and benign atypical cells in bile cytology that are used to differentiate benign and malignant cells.

Materials and Methods: Bile cytology specimens were collected by aspiration. 62 cases of well-differentiated adenocarcinoma with histological diagnosis and 19 benign cases were examined with 44 cases of moderately/poorly differentiated adenocarcinoma and 15 normal cases as controls. Cell counter software was used for objective evaluation of (1) cluster size, (2) cluster margin, (3) internuclear distance, (4) overlap, (5) nuclear shape, (6) anisonucleosis, (7) nuclear size, (8) nuclear/cytoplasmic ratio, (9) distribution of chromatin, and (10) nucleoli size in individual cases; evaluation of (11) necrosis and (12) vacuolization were binary, the presence or absence of these findings. A Kruskal-Wallis test was used to compare the 4 groups of well-differentiated adenocarcinoma, moderately/poorly differentiated adenocarcinoma, benign, and normal cells ($P < 0.05$). A Steel-Dwass test was used to select significant findings for differentiating well-differentiated adenocarcinoma from benign atypical cells ($P < 0.05$).

Results: Cluster margin, nuclear shape, distribution of chromatin, internuclear distance, and overlap were significantly different between the well-differentiated adenocarcinoma group and benign group in the Kruskal-Wallis test and Steel-Dwass test, sug-

gesting that quantification can be used to extract significant findings.

Conclusion: The differentiation of well-differentiated adenocarcinoma from benign atypical cells has often resulted in inconsistent diagnosis due to the ambiguity of differentiation criteria and subjective evaluation by observers. However, cytological evaluation could be more accurate by quantitative analysis of statistically significant findings.

Disclosure of Interest: None declared.

P-194

Cytodiagnostic Challenge: Carcinoma or Reactive Atypia? EUS FNA of Bile Duct in the Field of Parasitosis Giardia Lamblia

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Objectives: We introduce a case of a 67-year-old man with a confirmed stenosis of distal ductus choledochus. Due to a suspected neoplasia Endoscopic Ultrasound Fine Needle Aspiration (EUS FNA) was indicated. After our diagnostic conclusion a partial duodenopancreatectomia was performed. The presentation aims to introduce a rare and diagnostically difficult cytologic case report.

Material and Methods: Two EUS FNA punctures were performed from which 16 cytology smears were prepared, 14 were stained using Diff. Quick method, 2 were stained using PAS method, cytoblock was diagnostically fruitless. The bioptic preparations obtained from the partial duodenopancreatectomy were standardly embedded in paraffin and stained using haematoxylin – eosin method.

Results: Within the on-site cytology the smears were assessed as cytologic atypias, malignity cannot be excluded. When diagnosing completely processed material the trophozoites of Gardia lamblia were found on an inflammatory background. The blood cytology smears showed groups or isolated lamellae of cylindrical epithelium with atypias of various grades. The inflammatory background and numerous trophozoites of the parasite were observed also near the elements which fulfilled the morphological criteria of malignity. The patient consequently underwent partial duodenopancreatectomy, with a result of biopsy examination – adenocarcinoma of pancreas grade 2.

Conclusion: In the Czech Republic giardiasis is the most common intestinal disease caused by a parasitic protozoa. The finding of atypical epithelial cells which more or less fulfil the criteria of malignity and an inflammatory background with numerous trophozoites may cause a diagnostic uncertainty and contradiction. This case report is interesting due to fact that apart from the reactive changes of elements were in the smears present also malignant cells of adenocarcinoma of pancreas. Parasitosis is, in this case, assessed as a rare superinfection. However, there are papers which relate chronic parasitic infection with malignant tumours. A detailed development of the disease, diagnostic algorithm and figure amendment will be a part of poster presentation.

Disclosure of Interest: None declared.

P-195

Atypical Diagnostic Category of Bile Cytology in Pancreatobiliary Carcinoma and Benign Diseases

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Objectives: Bile cytology is still useful tool in evaluation of the pancreatobiliary diseases. In clinical practice, 'atypical' diagnostic category includes from favor benign to suspicious of malignancy. The aim of this study was to investigate the characteristics of 'atypical' cytology and increase the sensitivity and specificity of this diagnosis.

Materials and Methods: We retrospectively evaluated 303 bile cytology specimens from 115 cases of surgically resected pancreatobiliary cancers or benign diseases from 2010 to Dec, 2015 at Jichi Medical University Hospital. Cytological diagnoses were classified as negative, atypical and malignant.

Results: 51 of 91 carcinomas were cytologically diagnosed as malignant; 41 of 64 (64.1%) in bile duct carcinomas, 4 of 7 (57.1%) in gallbladder carcinoma, 5 of 16 (31.3%) in pancreatic ductal carcinoma and 1 of 4 (25%) in ampullary carcinoma. There were 25 'atypical' category in 91 carcinoma and 5 of 24 benign diseases including sclerosing cholangitis, chronic cholecystitis. Specificity of bile cytology was 79.2% with no false-positive case. The characteristics of 'atypical' cytology in carcinomas were 1) small amount of abnormal cells, 2) marked degenerative changes of nuclei and 3) lots of benign cell clusters in background, and in benign diseases were 1) existence of several papillary clusters, 2) irregularity of nuclear arrangement and 3) degenerative changes of cells.

Conclusion: The reasons of 'atypical' cytology were insufficient specimens and/or ambiguous criteria of carcinoma cells. It needs more objective criteria based on the comparative review both of cytology and histopathology.

Disclosure of Interest: None declared.

P-196

Cytomorphological Evaluation and Sub-Categorization of Hepatoblastoma

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Background: Hepatoblastoma (HB) is a rare pediatric solid tumor constituting approximately 1% of childhood cancers. Liver biopsy theoretically increases the risk of needle track tumor seeding or dissemination and is rarely performed. Cytology smears are often the only tissue available for evaluation prior to definitive therapy. Moreover, in patients with atypical imaging findings, normal alpha-feto protein (AFP) level or non-responsive to standard neoadjuvant chemoregimen, fine needle aspiration cytology (FNAC) becomes even more important.

Objectives: We undertook this study to evaluate the cytological features of mostly treatment naive HB and correlate with histology wherever available.

Materials and Methods: Ultrasound guided FNAC smears were obtained from 31 children (25 males and 6 females), aged 8 months to 11 years and stained with May-Grunwald Giemsa and Papanicolaou stain. Post-chemotherapy surgical resection specimens were available in 18 patients.

Results: All the patients were diagnosed with epithelial HB on cytology. Three dimensional clusters, few traversed by endothelial cells, was the predominant architecture followed by loosely cohesive sheets, and less commonly acini/pseudorosette. We endeavoured to further subtype the tumors, into pure fetal (12), embryonal (8), admixture of both (8), and small (3) cell types. The fetal cells were medium to large sized with abundant cytoplasm, uniform nuclei and usually single prominent nucleoli, recapitulating fetal hepatocytes. Embryonal cells had higher nucleo-cytoplasmic ratio, exhibited anisonucleosis and multiple small peripherally located nucleoli. The small cell type proved the hardest to diagnose conclusively as HB. HepPar1 and AFP immunostaining helped to rule out other malignant small round cell tumors (MSRCTs) like neuroblastoma, blastema-rich Wilms tumor, primitive neuroectodermal tumor and lymphoma. Mesenchymal components identified in few smears comprised of fragments of spindle shaped fibroblasts. No myoid, chondroid, osseous tissue or teratomatous element was noted. Extramedullary hematopoiesis (EMH) prominently featured in the background of all but six smears. Pure epithelial HB was the final diagnosis in thirteen surgical resections specimens while five had mixed epithelial and mesenchymal type of HB. The cytological subtyping correlated well with the corresponding histology.

Conclusions: HB can be conclusively diagnosed and appropriately sub-categorized by cytology in most of the patients. Immunohistochemistry is a useful adjunct in difficult cases by ruling out other common MSRCTs. Also EMH is an important clue to the diagnosis and must be diligently looked for in tumors with diagnostic dilemma.

Disclosure of Interest: None declared.

P-197

Fine-Needle Aspiration Cytology of Hepatic Angiosarcoma with Anastomosing Meshwork Scaffolding Hepatic Tissue Plates and Its Histopathological Correlation: A Case Report

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Objectives: Hepatic angiosarcoma is a rare hepatic malignant tumor. Cytological diagnosis of this tumor by fine-needle aspiration (FNA) cytology is difficult due not only to its various cytomorphologic features but also clinical rarity. Vasoformative features, such as pseudoacini, branching pseudocapillary structure, and intracytoplasmic lumina, are clues to achieve the correct diagnosis. But these features are not always present.

Case: A 60-year-old male with end-stage renal disease. Multiple infiltrative hepatic tumors were noted accidently by imaging study. Liver FNA cytology and subsequent biopsy were done.

Result: The cytological feature shows anastomosing meshwork wildly scaffolding architecturally intact hepatic tissue plates. Histopathological biopsy shows neoplastic endothelial cells infiltrating hepatic parenchyma and dissecting sinusoids, which is compatible with its cytological feature.

Conclusion: The above feature may be a clue for diagnosis of angiosarcoma by FNA cytology.

Disclosure of Interest: None declared.

P-198

Post-Transplant Hepatic Lesions: Fine Needle Aspiration Diagnosis

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Objectives: To describe the fine needle aspiration [Fna] cytology findings of infectious lesions in the post-tranplant liver and to emphasize the importance Fna diagnosis

Materials and Methods: Twenty seven patients with post-transplant lesions were subjected for fine needle aspiration diagnosis. Two air-dried smears were stained with Wright-Giemsa and two ethanol [70%] fixed smears were stained with Papanicolaou methods.

Results: Four cases were diagnosed as candidiasis, which showed numerous septate hyphae and spores and stained positively with periodic acid Schiff. Eight cases were diagnosed as mucormycosis, which showed many small, broad non-septate hyphae with right angle branches in a dirty back ground and stained negatively with periodic acid Schiff. Three cases were diagnosed as aspergillosis, which showed numerous slender, thin, septate hyphae

with acute angle branches. The hyphae stained positively with periodic acid Schiff.

Ten cases were diagnosed as actinomycosis, which showed many purplish deposits containing numerous filaments and granules in the back ground of neutrophils. The filaments and granules stained positively with periodic acid Schiff. Two cases were diagnosed as cytomegalic virus infection, which showed many large cells with intra nuclear inclusions [owl-eye].

Conclusion: Morphology of infectious agents were very clear in the cytology smears and helped for quick diagnosis and for early treatment without need of surgical intervention.

Disclosure of Interest: None declared.

P-199

Stromal Cell-Derived Factor 1 in Tumour Microenvironment Contributes to the Promotion of Colorectal Cancer

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Objectives: In tumor microenvironment, SDF-1 induced by CAFs that may promote tumor migration and invasion of CRC cells; and during this process; the CRC cells may also obtain capabilities of epithelial-mesenchymal transition, enrich properties of cancer stem cell, drug resistance, and anti-apoptosis. The interaction between SDF-1, taken by receptor (CXCR4) of CRC cells, generating SDF-1/CXCR4 loop, may induce CRC cells via paracrine and/or autocrine route(s) eventually enhances tumor aggressiveness leading to recurrence and metastasis.

Materials and Methods: The clinical CAFs (Cancer-associated fivroblasts) and NFs (normal fibroblasts) will be taken from patients of CRC by primary culture. First, we will check the characteristic of NFs and CAFs by detecting the CAF markers which are α-SMA (α-smooth muscle associated protein), vimentin. Second, to investigate the interaction between colon CAFs/NFs and colon cancer cells, CAFs/NFs and colon cacner cell lines will be cultured by organotypic raft culture. Third, to analyze invasion migration ability of colon cancer cell lines after cells be treated by the supernatant of CAFs/NFs by RT-PCR, western-blot, flow cytometry, and EMT markers. Finally, the expression level of SDF-1 and CXCR4 will be detected in CAFs/NFs from primary and metastasis CRC.

Results: Based on our present study, we found that the expression of SDF-1 and its receptor C-X-C chemo receptor type 4 (CXCR-4) are stronger when co-cultured with CAFs than with NFs. In addition, ELISA assay also validates the presence of SDF-1 that is responsible for the crosstalk between fibroblasts and CRC cells via the paracrine effect. Furthermore, the mediator SDF-1 not only triggers Epithelial-Mesenchymal-Transition (EMT), showing upregulation of EMT markers in RNA and proteins levels, but also improves the capabilities of migration and invasion. CRC cells treated with recombinant SDF-1 increased capabilities of sphere formation in ten days. Further studies include evaluations of the properties of cancer stem cells (CSCs) and drug resistance. Meanwhile, validates via in vivo model and clinical patients.

Conclusion: Our results conclude that CAFs promote cancer invasiveness via paracrine and autocrine effects on microenvironmental SDF-1 signalling, and suggest that SDF-1 is a potentially biomarker and contributes to the expression of cancer stem cells' properties, and can be further considered in therapeutic strategies for the treatment of patients with CRC.

Disclosure of Interest: None declared.

P-200

Mucinous Balls Tangling with Spindle Cells in the Ascites of Pseudomyxoma Peritonei

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Objectives: In order to find cytological features of ascites in a patient with pseudomyxoma peritonei (PMP).

Patient and Methods: The patient is the 7th decade male, and the smears and cell block of PMP ascites were investigated.

Results: He was referred to the hospital with abdominal distension. The ascites was gelatinous, colorless to yellowish on gross examination, and needle aspirate smears showed many mucinous balls wrapped by spindle cells, in addition to histiocytes and mesothelial cells. The cell block showed a few MUC2 positive adenocarcinoma cell clusters. The diagnosis of PMP was made, and cytoreductive surgery was performed. The cystic diverticulum involved by mucinous tumor cells in the sigmoid colon was an origin of PMP.

Conclusion: The mucinous balls wrapped by spindle cells in the smear and mucinous immunostaining of the cell block may be effective for the diagnosis of PMP.

Disclosure of Interest: None declared.

P-201

Solid Pseudopapillary Neoplasm: Rare Presentations of an Uncommon Entity

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Objectives: Solid Pseudopapillary Neoplasm (SPN) is an uncommon epithelial neoplasm of low malignant potential that occurs almost exclusively in young women. Our purpose is to discuss some infrequent characteristics of this entity, based on 2 SPN cases from our archive.

Materials and Methods: Case No 1 was about a 22 mm lesion in the pancreatic body of a 52 year old man and Case No 2 about

a 58 mm lesion in the pancreatic head of a 44 year old woman. EUS-FNA with accompanying rapid on site evaluation (ROSE) was performed in both cases. Romanowsky and Papanicolaou stained conventional smears, liquid based cytology slides and formalin fixed cell blocks were prepared. Immunocytochemistry was applied on the cell blocks.

Results: Both cases were highly cellular with a typical 'solid cellular pattern' and shared a similar cytomorphology: a monomorphic population of cells arranged both singly and in groups, some of which depicting a pseudopapillary architecture, was accompanied by a background of bare nuclei, debris and hyaline globules. Nuclei had a round to oval shape, a finely granular chromatin and a micronucleoli. Some of them appeared with indentations or grooves. Cytoplasm was delicate and revealed at times a perinuclear vacuole. Interestingly, cercariform cells were noticed too. Immunocytochemistry of the Cases No 1 and No 2 showed: nuclear β-catenin(+), E-cadherin(–), CD10(+), CD56(+), Vimentin (+), PR(+) and chromogranin(–). Synaptophysin was negative in Case No 1 and positive in Case No 2.

Conclusion: SPN is most commonly diagnosed in young women, with a mean age of 28 years. Case No 1 involves a 52 year old man and Case No 2 a 44 year old woman. SPN is not in the top list of the differential diagnosis regarding pancreatic lesions in these ages.

Neuroendocrine differentiation in SPN cells is commonly encountered and neurosecretory granules have been observed in their cytoplasm with electron microscopy studies. This can cause problems separating it from its main differential diagnostic entity, the Pancreatic Endocrine Neoplasm (PEN). They both share expression of CD56 and, to make things worse, synaptophysin and chromogranin can be positive in 26% and 15% of the SPN cases respectively. Mutations in the CTNNB1 gene that encodes the β-catenin protein result in a positive nuclear immunostain, a very helpful feature to confirm SPN. Other markers such as E-cadherin membranous negativity, paranuclear dot-like positivity for CD99 and positivity for CD10 and galectin 3 can be combined with the architectural, cytomorphologic and background features described above to support a diagnosis of SPN.

Disclosure of Interest: None declared.

P-202

Reflux Esophagitis: Brushing Cytology Findings

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Objectives: To describe the cytologic findings of reflux esophagitis and discuss the differential diagnoses. Also to assess the cytologic features of reflux esophagitis and to determine if it is possible to diagnose it based on brushing cytology smears.

Materials and Methods: Brushing cytology smears of 107 cases of reflux esophagitis were studied. All cases were histologically confirmed as reflux esophagitis. The ages ranged between 40–60 years old, 97 were male and 10 were female. The brushing cytology smears were obtained before biopsy and were stained with Wright-Giemsa and Papanicolaou methods.

Results: Endoscopic findings of all cases revealed hyperemia at distal esophagus. Clinically suspicious for viral and fungal infections, reflux esophagitis and malignancies. The cytology smears revealed hemorrhage, isolated and clusters of squamous cells, good number of neutrophils, eosinophils, lymphocytes and plasma cells. Fungal elements and viral inclusions were not seen. Malignant cells were not seen.

Conclusion: Cytologic findings of reflux esophagitis were rarely described. It is very difficult to diagnose reflux esophagitis on cytology findings alone without clinical information. Viral, fungal, Barrett's esophagitis, collagen vascular diseases and idiopathic eosinophilic esophagitis should be considered in the differential diagnosis.

Disclosure of Interest: None declared.

P-203

Fine Needle Aspiration Cytology of Gastrointestinal Mesenchymal Tumors

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Objectives: Gastrointestinal mesenchymal tumors (GIMTs) are relatively uncommon neoplasms. Most of GIMTs can be classified into three types; gastrointestinal stromal tumor (GIST), leiomyoma, and schwannoma. Although leiomyoma and Schwannoma are benign tumors, GIST is malignant. Generally, it is not always easy to distinguish GIST from other GIMT. The aim of this study is to examine cytological features of specimens from GIMTs taken by Endoscopic Ultrasound Fine Needle Aspiration (EUS-FNA).

Materials and Methods: We collected and reviewed Papanicoloau-stained specimens of 8 leiomyomas, 3 gastrointestinal schwannomas and 10 spindle cell type GISTs from the cytology files of our hospital.

Results: Cells from leiomyomas were composed of cohesive, paucicellular pattern of spindle cells with light green-colored cytoplasms. Fascicular arrangements were seen in large clusters. Those from schwannomas were moderately hypercellular, showing both loose and dense cellular sheets of spindle cells. Each tumor cells had scanty cytoplasm and bland nuclei, but focal nuclear atypia was common. Fascicular arrangements were seen in large clusters, together with isolated cells. Cells from GISTs showed the most hypercellular pattern compared to the other 2 tumors. Spindle tumor cells composed of storiform/fascicular pattern. Occasionally palisading arrangements were seen. Isolated tumor cells were rarely observed. In all cases, tumor cells showed low mitotic counts.

Conclusion: Not surprisingly, not only morphological features but also immnohistochemical results appear to be important for making a diagnosis. However, cytologic specimens taken by EUS-FNA may preserve three-dimensional clusters reflecting tissue structures, and therefore it will be more useful for accurate diagnosis.

Disclosure of Interest: None declared.

P-204

A Case of a Peripancreatic Paraganglioma: A Diagnostic Challenge on Fine Needle Aspiration (FNA)

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Introduction: Paragangliomas are rare tumors affecting 1–2/100,000 adults per year. Extra-adrenal paragangliomas presenting as a pancreatic/peripancreatic mass are even rarer and pose a diagnostic challenge on FNA with a high rate of diagnostic error (50% in case reports). Due to non-specific clinical and radiographic findings often mimicking more common pancreatic neoplasms, the diagnosis relies solely on the cytopathologist's expertise and level of suspicion. Incorrect diagnoses rendered by FNA include a variety of epithelial and mesenchymal tumors, both benign and malignant.

Case Report: We present a case of paraganglioma in a 58 year-old female presenting with right-sided abdominal pain. Imaging studies demonstrated a 6 cm mass in the right upper quadrant causing mild dilation of the pancreatic duct, and either arising from the pancreatic head or abutting it posteriorly. Endoscopic ultrasound guided FNA of the mass, designated at the time as a 'pancreatic head mass,' showed a spindled/epithelioid cell proliferation arranged singly, in loose clusters, and focally around arborizing vessels with occasional rosette-like structures. The tumor cells were uniform with a moderate amount of cytoplasm and dense nuclei. There was no nuclear atypia, mitosis, or prominent nucleoli. A wide differential diagnosis was considered including GIST, paraganglioma, solid-pseudopapillary neoplasm, acinar cell carcinoma, pancreatic endocrine neoplasm and well differentiated adenocarcinoma. Synaptophysin, chromogranin and Beta catenin (cytoplasmic only) were positive; CD117 and S100 were negative. The final FNA diagnosis was a neuroendocrine neoplasm, either a paraganglioma or pancreatic endocrine neoplasm. Surgical resection confirmed this neoplasm to be a retroperitoneal peripancreatic paraganglioma.

Conclusion: This case study highlights the difficulties in the accurate preoperative diagnosis of paraganglioma in the peripancreatic region, and emphasizes the importance of keeping this entity in the differential diagnosis of a pancreatic/peripancreatic mass.

Disclosure of Interest: None declared.

Lymph Nodes / Blood

P-205

CD30-Positive ALK-Negative Pleomorphic Large B-Cell Lymphoma with an Unusual Cytomorphology Mimicking Metastatic Carcinoma in Touch-Imprint Cytology – A Case of Potential Pitfall

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Introduction: In lymph node cytology, atypical cohesive cellular aggregates are basically considered to be a metastatic lesion, such as poorly differentiated adenocarcinoma. Rare exceptional cases of lymphoma, however, can demonstrate a perplexing cytomorphology indistinguishable from metastatic carcinoma, which often misleads us into an incorrect cytodiagnosis. We report herein a rare case of malignant lymphoma showing a confusing cytomorphology mimicking metastatic carcinoma in touch-imprint cytology.

Case: A 54-year-old Japanese male visited our hospital with a chief complaint of right inguinal lymphadenopathy. Under a preliminary clinical diagnosis of malignant lymphoma, he underwent the lymph node excisional biopsy and the touch-imprint cytology was also performed.

Cytological Findings: Papanicolaou staining revealed many cohesive aggregates of large polygonal cells with medium to large atypical lymphocytes distributed in the background. Giemsa staining demonstrated the aggregates with dense and deep basophilic cytoplasm indicating a potential diagnosis of metastatic carcinoma rather than a nodal large/pleomorphic cell lymphoma.

Histopathological Findings and Diagnosis: The basic histological structure of the lymph node was completely effaced and replaced mainly with atypical large polygonal/pleomorphic cells. In the marginal sinuses, sheet-like cell aggregates were easily detected and our primary impression was a metastatic undifferentiated carcinoma. With further multidisciplinary approaches including flow cytometry, immunohistochemistry, chromosomal analysis and electron microscopy, our final diagnosis was a rare variant of diffuse large B-cell lymphoma: CD30+/ALK– pleomorphic large B-cell lymphoma.

Discussion: This case is considered to be a subtype of diffuse large B-cell lymphoma previously called anaplastic large cell lymphoma of B-cell type (B-ALCL) or B pleomorphic lymphoma. This subtype often shows epithelioid cohesive aggregates of tumor cells in the marginal sinuses of lymph node typically observed in various types of metastatic carcinoma. Conventional ALCL of T/null cell type and rare ALK+ large B-cell lymphoma (WHO 2008) are also known to demonstrate the similar findings and can even show cytokeratin positivity. Although our case is a rare subtype, it is a

potential pitfall, however, in daily cytology practice and this variant should be taken into differential diagnoses of metastatic carcinoma in lymph node cytology.

Disclosure of Interest: None declared.

P-206

BCL2-JH Assessment in Non-Hodgkin Lymphoma Cells on FTA Cards

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Objective: BCL2-JH rearrangement in non-Hodgkin lymphoma (NHL) is detectable in 90% of follicular lymphoma (FL) and in 20% of diffuse, large, B-cell lymphoma (DLBCL). BCL2-JH evaluation may be useful to the clonality assessment of NHL and to the classification of FL and DLBCL on cytological samples. NHL cells, collected by fine needle cytology (FNC), may be stored on FTA filter paper cards that immobilize and stabilize nucleic acids and can be stored at room temperature. This study evaluates whether BCL2-JH assessment may be performed on NHL cells collected by FNC and stored on FTA cards.

Materials and Methods: 13 FL, 7 DLBCL, 31 unspecified NHL and 44 reactive lymph node (BRH) FNC cells were stored on FTA cards and DNA extraction was performed on 2 punched disks for each case. Fifty nanograms of DNA were used to amplify exon 14 of Jak2 gene to assess the DNA integrity and to detect the BCL2-JH rearrangement by two distinct multiplex-PCR tubes. BCL2-JH status was then compared to the BCL2 phenotypic expression, evaluated by immunohystochemistry (IHC), on corresponding cell blocks or histological controls.

Results: BCL2-JH rearrangement was observed in 8 out of 13 FL (61%), in 5 out of 7 DLBCL (71%) and in 10 out of 31 (32%) unclassified NHL. These data were concordant with the IHC results in all the cases.

Conclusions: BCL2-JH rearrangement can be successfully detected on FNC, FTA cards stored cells and the obtained data are consistent to those of IHC.

Disclosure of Interest: None declared.

P-207

Cytological Diagnosis of Non Hodgkin Lymphoma in situ, Is It Possible?

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Objective: Intrafollicular neoplasia/in situ follicular lymphoma is a newly defined pathologic entity included in the 4th edition of the World Health Organization 2008. classification of the lymphoid neoplasms. New WHO classification defined the intrafollicular neoplasia/in situ follicular lymphoma as architecturally normal-appearing lymph nodes and other lymphoid tissues that have one or more follicles that demonstrate bcl-2 overexpressing centrocytes and centroblasts, with or without a monomorphic cytologic appearance suggestive of follicular lymphoma.

Material: A 45-year-old female presented in the 2006. with enlarged right preauricular lymph node, detected incidentally during an episode of fever. Full blood count and serum lactate dechidrogenase level were normal. The patient had only sweating. FNA was performed and majority part of cytological smear showed lymphoid cells resembled mature small lymphocytes, centrocytes and centroblasts, but the rest of the smear showed huge atypical lymphoid cells. Cytological finding was suggestive of non Hodgkin lymphoma, follicular type. Ultrasonography showed that the node was single, hypoehogenic, measured 0.5x1 cm in diameter. Immunophenotypisation of lymph node lymphocytes revealed clonal expression of kappa chains in 40% of B lymphocytes. The established diagnosis was B non Hodgkin lymphoma, follicular type. The lymph node was excised. Histologically, the most germinal centers of the lymphoid follicles showed tingible body macrophages and an intermixed population of centrocytes and centroblasts. On the basis of immunohistochemically performed CD20, CD3, Bcl-2, Bcl-6, CD10, MUM-1, CD57, TIA, CD30, CD15, EMA, EBV-LMP and granzyme-B, the diagnosis of florid hyperplasia was established. The patient has been maintained on regular USG follow-up for last 9 years with a wait-and-watch policy.

Conclusion: The clinical significance of in situ follicular lymphoma is that some patients are found to have follicular lymphoma elsewhere, either before or simultaneously, while some develop overt follicular lymphoma later, or no evidence of follicular lymphoma is seen in the others. As defined in the WHO classification of lymphoid neoplasms, it is impossible to diagnose in situ follicular lymphoma based only on histologic findings without additional immunohistochemical staining for bcl-2, flow cytometry, cytogenetic FISH analysis, PCR etc. Incidence of in situ follicular lymphoma is very low, so awareness of the early neoplastic lesions of the lymphoid tissue is needed. Morphology (histology or cytology) could not alone make the diagnosis of in situ lymphoma, so additional methods are mandatory.

Disclosure of Interest: None declared.

P-208

T-Cell Large Granular Lymphocytic Leukemia (TLGL)

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Introduction: T-cell large granular lymphocytic leukemia (TLGL) is caused by clonal proliferation of cytotoxic (CD8+) T cells. In most cases it is indolent and associated with mild to moderately stable lymphocytosis, neutropenia, splenomegaly and occasionally anemia, while lymphadenopathy is very rare. TLGL demonstrates a strong association with autoimmune diseases, es-

pecially rheumatoid arthritis. The male:female ratio is approximately one and the majority of cases occur in the 38–72 yr age group. Diagnosis is typically based on high number of morphologically characteristical lymphoid cells and findings of an abnormal immunophenotype by flow cytometry. Median survival of more than 10 years is typical for these patients. Treatment depends on whether the person has symptoms or if the disease si causing problem. Because of its relatively indolent clinical behavior, observation is often an appropriate therapy. The drugs most commonly used to treat TLGL leukemia are methotrexate, cyclophosphamide and cyclosporine with or without prednisolone.

Case Report: A 53-year-old male was admitted to hospital because of abdominal pain. Blood examination revealed mild mycrocitic anaemia and multiplied lactate dehidrogenase (LDH) level. Abdominal ultrasound revealed splenomegaly of 16 cm, with no lymphadenopathy. FNA of bone marrow revealed hypocellular marrow with 50% of atypical lymphoid cells which were partially positive on acid phosphatase stain. There were 81% of atypical medium sized granular lymphocytes with irregularly shaped nuclei in the peripheral blood, so the cytological diagnosis was lymphoproliferative process. Bone marrow biopsy showed nodular and interstitial proliferation of small, partially atypical T lymphocytic cells positive on CD2, CD3, CD5, CD8, Granzyme and TIA and negative on hairy cell markers, CD10, MUM 1, bcl 1, CD4 and CD56. The finding was consistent with TLGL. The patient is due the splenomegaly treated with cyclophosphamide and gradually reduced dose of corticosteroids, leading to the withdrawal of splenomegaly.

Conclusion: TLGL is uncommon, but probably underdiagnosed malignancy, which is from 2008. in WHO classification recognized as a well defined clinical entity, so the effort has to be made in the identification of patients having said disease.

Disclosure of Interest: None declared.

P-209

A Case Report of Burkitts Lymphoma Diagnosed on Bone Marrow Aspirate Cytology with Leukemic Conversion from Non-Endemic Region

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Introduction: Burkitt's lymphoma is rare in India and constitutes only 0.7% of total childhood solid tumor malignancies. It is endemic in Africa and shows strong association with Epstein -Barr virus, Human Immunodeficiency virus and malaria. The usual presentation of Burkitt's lymphoma in endemic region is maxillo-facial involvement with lymphadenopathy while sporadic cases shows abdominal involvement. Leukemic conversion is considered to be extremely rare in Burkitt's lymphoma and till date only few cases have been reported in literature. The present case is being reported due to its unusual presentation with absence of any abdominal or facial mass but with bone marrow involvement and associated with an important feature of leukemic conversion in immunocompetent patient from non-endemic area.

Case Report: A twelve year female child presented with off and on fever, progressive pallor and generalized weakness for last 6 months. Her abdominal ultrasonogram showed hepatosplenomegaly and patient was non-reactive for human immunodeficiency virus. Her complete hemogram report showed pancytopenia with hemoglobin of 46 gm/L, hematocrit 13.44%, mean corpuscular volume 84.69fL, total leukocyte count of $3.67x10^9$/L and platelet count of $16.2x10^9$/L. Her differential count on peripheral smear showed 30% atypical lymphoid cells which were large with scant to moderate agranular light blue cytoplasm, coarse clumped chromatin and inconspicuous nucleoli. Few of these blasts showed cytoplasmic as well as nuclear vacuolations. In view of pancytopenia and atypical cells, bone marrow examination was advised which revealed complete effacement of the marrow with blasts of same morphology as in peripheral blood. Peripheral blood was subjected to flowcytometry which showed positivity for CD45 (bright), CD19, CD10, CD43 and negative expression of CD3, CD5, and CD20, BCL-2, CD25 and kappa/lambda. Immunohistochemitry on bone marrow biopsy showed positivity of Ki-67 in more than 95% blasts. In view of above clinical and laboratory findings diagnosis of Burkitt's lymphoma with leukemic conversion was considered. However, no cytogenetic and molecular analysis was done due to patient's reluctance and financial constraints. The condition of patient deteriorated rapidly and patient succumbed to her illness with a week.

Conclusion: Burkitt's lymphoma, although rare must be considered even in non-endemic region which may present without any abdominal or facial swelling in immunocompetent patient and may be diagnosed only on bone marrow examination. In addition, the case also highlights an important feature of leukemic conversion of Burkitt's lymphoma which is rarely reported in literature.

Disclosure of Interest: None declared.

P-210

Cancelled

P-211

Value of a Modified Procedure of Fine Needle Aspiration and Cell Blocks Preparation in the Diagnosis of Metastatic Nasopharyngeal Carcinoma in Lymph Nodes

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Objectives: To testify the sensitivity and final diagnosis rate of our modified procedure of the fine needle aspiration (FNA) biopsy method and the cell block's preparation. The metastatic nasopharyngeal carcinoma of lymph nodes was employed as the candidate in this purpose. In another more purpose, we also testified whether it could benefit the immunohistochemistry and in situ hybrid based on this cell block's procedure.

Materials and Methods: In methodology, the FNA procedure was sampled using our patented aspirators (Youyi-style aspirator) in a pencil-grip manner. In this study the cell block was prepared at the same time and was simplified protocol rather than a second operation. With this aspirator, more FNA materials were sampled and thus easily coagulated to be a conglobation in 95% alcohol and then replaced the fixation by 10% formalin. Except the cell block, cell smears were also prepared for routine stain. The IHC and EBER tests were based on the cell block sections. From 2009 to 2015 A.D. 628 patients with cervical lymph adenitis or lump were reviewed in our FNA clinic and most of neoplastic cases had following surgical biopsies. The final diagnosis of FNA was based on the morphology of cell smears/cell block sections and a panel of IHC combining EBER ISH results. The evaluation of the sensitivity and final diagnosis rate of FNA in lymph node of metastasis nasopharyngeal carcinoma in the cohort were compared with the surgical biopsy, radiology and clinical follow-ups.

Results: A amount of 31 metastatic nasopharyngeal carcinoma was diagnosed 20 of all 31 cases showed the characteristics of undifferentiated type carcinomas and 11 cases shared the morphology with differentiated type carcinoma. The tumor cells of most lesions (96.8%, 30/31 cases) which shown positive expressed in CK5/6, P63, Ki-67 and EBER. The LCA and CK7 were all negative (100%, 31/31 cases). The sensitivity and the final diagnostic rate were the lowest in the microscopy cell smears simply (90.3%, 28/31 and 74.2%, 23/31 cases) and the middle in the microscopy smears combined cell block sections (93. 5%, 29/31 cases and 77.4%, 24/31 cases) and the highest in the microscopy smears combined cell block sections with tests of IHC and EBER (96.8%, 30/31 and 96.8%, 30/31 cases), respectively.

Conclusion: Our patented aspirators in a pencil-grip operation manner, which improve adequacy samples to make cell blocks. The simplified cell blocks preparation profited not only the morphology but also the IHC and in situ hybridization. The FNA combined cell block procedure might improve he practical significance as surgical biopsy in the diagnosis of lymph node lesions, whether lymphoma or metastatic nasopharyngeal carcinoma presented.

Disclosure of Interest: None declared.

P-212

Cytological Diagnosis of Neck Lymph Nodes Pathology

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Introduction: Immunocytochemistry method of research, including fluorescent immunocytochemistry can significantly help in determining the nature of lymph nodes.

Purpose: To identify opportunities immunocytochemistry method of research, including fluorescent immunocytochemistry in the diagnosis of lymph node.

Materials and Methods: Method immunocytochemistry and fluorescent immunocytochemistry studied 104 cervical, 12 podcheslyustnyh lymph nodes. The study used a broad panel of antibodies ('Dako'): to epithelial antigen Ber EP4 FITC fluorescently label. total cytokeratin, EMA, epithelial antigen Ber EP4, vimentin, desmin, CK7, CK20, CK5/6, CA125, CEA, ER, RP, hromagranin A, synaptophysin, HBME-1, TTF-1, thyroglobulin, calcitonin, GCDFP-15, WT-1, cdx2, CD45, CD20, CD3, CD10, CD5, CD23, CD15, CD30, Cyclin D1, CD68, bcl2, Ki67, HMB45, S100, melan A, p63, NSE, c-erbB2, PSA.

Results of the Study: With immunocytochemistry and fluorescent immunocytochemistry in the diagnosis of lymph node following tasks: metastatic lymph nodes were analyzed to clarify the histogenesis of primary tumor – 38 (accuracy – 95%), clarification of the source of metastasis to lymph nodes in the presence of multiple malignant processes – 4 (histogenesis installed in all cases), determined the localization of malignant process in the presence of metastatic lymph nodes revealed no primary lesion – 15 (primary location is set to 67%), lymph node assay to confirm or exclude metastasis, including urgent intraoperative detection of metastatic lymph nodes – 22 (accuracy – 100%), differential diagnosis between lymphoma and cancer metastasis – 5 (100% accuracy), immunophenotyping lymphomas and Hodgkin's disease diagnosis – 32 (accuracy – 98%).

Conclusion: Methods immunocytochemistry and fluorescent immunocytochemistry Stuff reliable in the diagnosis of lymph node involvement. Particularly promising application of the method fluorescent immunocytochemistry urgent intraoperative diagnostic. Method is fast, reliable, does not require complicated preparation of drugs, the study takes 30 minutes.

Disclosure of Interest: None declared.

P-213

Improving the Yield of Cell Deposition Onto Glass Slides to Detect Rare Target Cells

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The cytocentrifugation protocol of the cell suspension onto glass slides requires maximal cell yield and highest recovery rate of living target cells to detect rare cells in a cell suspension. These deposited cells also need to retain their cellular integrity for cell or biomarker identification using antibodies and probes.

The common method using a cytocentrifuge with a standard protocol results in a 10–20% target cell recovery rate which is insufficient for retrieval and identification of rare cells like circulating fetal (e.g. prenatal diagnostics) or cancer cells (e.g. minimal residual disease).

Objective: A novel method is presented to deposit rare cells onto glass slides using an optimized cytocentrifugation protocol, which combines an aqueous based coating of the receiving glass slide with a modified acceleration and deceleration cytocentrifuge procedure.

Results: Two instruments (Thermo Scientific™ Cytospin™ 4 and Sakura Cyto-Tek® 2500) were used to evaluate the optimization of cytocentrifuge protocols using different acceleration and deceleration procedures in combination with either no coating or the novel slide coating. As a model, rare target cells were spiked

into human peripheral blood. Key performance indicators of comparison were cell yield and target cell recovery rate quantitatively determined using stains including hematoxylin and eosin, immunohistochemistry (IHC) and *in-situ* hybridization (ISH) with digital imaging.

Conclusion: The novel slide coating in combination with the optimized cytocentrifugation protocol significantly improves the cell yield and recovery rate of rare cells compared to common procedure.

Disclosure of Interest: All people listed on abstract are full-time employees of either Sakura Finetek USA or Aviva Biosciences Corp.

P-214

A Minimally Invasive Method (Liquid Biopsy) Enabling Detection of Rare Cells Using Cell Enrichment and Immunohistochemistry

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Whole blood, serving as a 'liquid biopsy', can provide a means of monitoring the status of a tissue-based event (e.g. pregnancy or cancer) using a minimally invasive needle stick of the patient. This minimally invasive method of monitoring disease status before, during and after treatment benefits both patient and physician.

Objective: To develop a protocol using a novel cell enrichment method combined with an immunohistochemical cell detection method, which may allow for a higher efficiency of rare cell recovery (greater than 30%).

Methods: Peripheral blood was spiked with rare target cancer cells. Cells were enriched by a novel chip based cell enrichment method. Cells were then stained using automated protocols (hematoxylin and eosin and immunohistochemistry (IHC)). Slides were visualized and analyzed using digital microscopy.

Results: Using 0.1 mL of whole blood containing approximately 10E8 total cells, the novel chip-based protocol enriched the rare target cells 100-fold and achieved a 50% recovery rate. The rare target cells were identified using a panel of relevant cell-specific biomarkers and IHC.

Conclusion: Using a novel target cell enrichment protocol in combination with IHC, liquid biopsy provides an alternative method to detect rare cells with potential utility in diagnosis and monitoring following the concept of personalized medicine.

Disclosure of Interest: All people listed on abstract are full-time employees of either Sakura Finetek USA or Aviva Biosciences Corp.

P-215

The Utility of Cytologic Features for the Diagnosis of Tuberculous Cervical Lymphadenitis

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Objective: This study was aimed to investigate an important cell morphological findings for the diagnosis of tuberculous cervical lymphadenitis and to evaluate their utility.

Materials and Methods: The subjects were 218 patients who underwent with fine-needle aspiration cytology and polymerase chain reaction (PCR) tests from January 2008 to July 2012. Compared with the results of PCR test and biopsy, the sensitivity and specificity were calculated and ROC analysis was performed in order to evaluate the utility of cell morphological findings of fine-needle aspiration cytology.

Results: The cell morphological findings such as epithelioid cell with caseous necrosis, epithelioid cell granuloma with caseous necrosis, and predominantly caseous necrosis were diagnostic for tuberculous cervical lymphadenitis. In a comparison of the cell morphological findings and a PCR test (which served as a reference), the sensitivity, specificity and area under curve (AUC) were 96.6%, 90.6%, and 0.936 respectively. In a comparison of the cell morphological findings and a biopsy (which served as a reference), the sensitivity, specificity and area under curve (AUC) were respectively.

Conclusion: We conclude that cell morphological finding of fine-needle aspiration cytology diagnosis is useful for the diagnosis of tuberculous cervical lymphadenitis without polymerase chain reaction (PCR) test or biopsy.

Disclosure of Interest: None declared.

P-216

Tularemia – An Underdiagnosed Disease?

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Background: Tularemia is a rare zoonosis caused by the Gram-negative rod-shaped bacterium Francisella tularensis. Reported mortality rate with appropriate antibiotic therapy is about 1%.

Methods: 12 Cases (7 fine needle aspirations, 3 lymph node biopsies, 1 joint fluid, 1 autopsy) with confirmed diagnosis of tularemia either by PCR or bacterial culture were retrieved from the archives of Institute of Surgical Pathology in oder to analyze morphological characteristics and to correlate with the clinical presentation.

Results: The typical clinical presentation was a cervical lymphadenopathy partially with grotesque enlarged nodes. One case was referred to EBUS guided FNA with suspicion of lung cancer because of FDG active lymph nodes in PET. A very unusual presentation was a prosthetic joint infection with Francisella tularensis.

Almost all patients had a history with animal contact (especially rodents like rabbits, rats or mice). Morphologically the specimens showed a broad range of patterns from acute purulent to granulomatous inflammation. Bacteria were morphologically not detectable. The diagnosis was established by PCR or bacterial culture.

Conclusions: Tularemia is a rare but serious illness which can be cured by appropriate antibiotic therapy. The clinical presentation might raise suspicion of malignancy because of fast growing tumor masses and enlarged lymph nodes. Although malignant neoplasms can be easily ruled out morphologically in adequate specimen the inflammatory pattern is not pathognomonic which might lead to under-diagnosis. Therefore awareness of this differential diagnosis and clinical-pathological correlation are crucial to initiate specific tests PCR, serology or bacterial culture in order to confirm the diagnosis of tularemia. FNA is an appropriate instrument for establishing the diagnosis and less invasive compared to lymph node biopsy.

Disclosure of Interest: None declared.

Soft Tissues

P-217

Cytopathological Spectrum of Epithelioid Sarcoma in a Series of 7 Uncommon Cases, Including Immunohistochemical Features with Special Reference to Loss of INI1/SMARCB1 Immunostaining, in Two Test Cases

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Objectives: To analyze cytomorphological features of epithelioid sarcoma (ES) in a series of 7 cases.

Materials and Methods: Over a 10-year-period, 7 cases of ES were retrieved from the hospital records in which fine needle aspiration cytology (FNAC) was performed.

Results: All 7 tumors occurred in males within age-range of 22–61 years, in forearm (3 cases), hand (2 cases), thigh (1 case) and inguinal region (1 case). FNAC was performed for metastatic lesions (5 cases), recurrent lesions (4 cases) and for a primary diagnosis (1 case). FNAC smears were mostly moderate to hypercellular comprising predominantly, polygonal (7 cases) and spindle cells (3 cases) with moderate to abundant cytoplasm, defined cell borders, vesicular nuclei and discernible nucleoli, arranged in loosely cohesive groups, non-overlapping clusters, as well as scattered singly. In five cases, tumors cells revealed paranuclear, intracytoplasmic inclusions, leading to rhabdoid appearance. Three cases revealed giant cells. Scanty metachromatic stroma was identified in smears of 4 out of 7 cases. On histopathology, two tumors were conventional type, three were proximal/large cell type and two tumors showed mixed features of conventional and proximal/large-cell type ES. By immunohistochemistry (IHC), tumor cells were positive for cytokeratin (CK) MNF116 (4/5), epithelial membrane antigen (EMA) (6/6), pan CK (AE1/AE3) (1/1), vimentin (3/3) and CD34 (6/6). Tumor cells were completely negative for INI1/SMARCB1 (2/2) and CD31 (4/4).

Conclusions: FNAC in cases of ES in our settings was mostly performed in cases of recurrent and/or metastatic lesions. Important cytomorphological features of an ES include presence of loosely cohesive, non-overlapping clusters of polygonal cells, including cells with rhabdoid features; admixed with spindle cells. Optimal IHC markers, including CK/EMA/AE1AE3 and CD34 are necessary for arriving at a correct diagnosis. Complete loss of INI1/SMARCB1 in tumor cells is useful in differentiating an ES from most carcinomas and certain other tumors that constitute as its differential diagnoses. Clinical correlation is imperative in all cases.

Disclosure of Interest: None declared.

P-218

Cytologic Features of Extraskeletal Myxoid Chondrosarcoma

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Introduction: Extraskeletal myxoid chondrosarcoma (EMC) is a rare soft tissue sarcoma with limited literature available on its cytological features. We report here one such case where a diagnosis of EMC was made based on fine needle aspiration cytology (FNAC).

Case: A 29 year-old male presented to our hospital with a slowly growing painful mass in the left calf, which was subjected to FNAC. Magnetic resonance imaging was suggestive of a malignant soft tissue neoplasm. The FNA smears showed cell fragments and cords of monotonous cells embedded in abundant myxoid stroma. Occasionally, linear trabecular and solid pattern was identified. The tumor cells showed a epithelioid or round shape with prominent nucleoi. A diagnosis of a myxoid sarcoma favoring an EMC was made. Subsequent wide marginal excision of the mass for histopathological examination confirmed this diagnosis.

Conclusion: EMC has a characteristic cytological features that are helpful in making a diagnosis in the appropriate clinical setting.

Disclosure of Interest: None declared.

P-219

A Case Report of Chordoma

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Objectives: Chordoma is the only malignant neoplasm derived from notochordal elements. This rare midline lesion usually occurs at one of the two ends of the axial skeleton. It is most commonly diagnosed between the ages of 50 and 60, and is more com-

mon in men. Patients can have symptoms for months to years and frontal headache is a common symptom before diagnosis.

Materials and Methods: A 62-year-old man had louder snoring and apnea during sleep in recent 6 months. He had neck and nuchal pain, and occipital headache occasionally. On physical examination, a bulging mass with smooth mucosal surface is noted over posterior wall of right oropharynx. Videoscopy showed a submucosal tumor over right oropharynx and hypopharynx, resulting in narrowing of airway and compression of right epiglottis. MRI revealed that the main lesion is located at C2 level, compromizing airway and cervical spinal cord, with destruction of cervical spine. Chordoma is considered less likely since the epicenter of main tumor is not at spine. The patient agreed to have needle biopsy of the prevertebral tumor through upper oropharynx.

Result: Fine needle aspiration from the prevertebral tumor showed a few large-sized epithelioid cells with abundant cytoplasm (may be the physaliphorous cells), embedded in delicate magenta-colored stromal substance. Nuclear atypia is evident. On sections of cell block preparation, these vacuolated tumor cells are arranged in small nests and cords, with extracellular and intracellular mucoid substance. Differential diagnosis includes mucinous adenocarcinoma, chondrosarcoma, chordoma, etc. Immunohistochemically, the tumor cells are positive for cytokeratin, EMA and S100, which are compatible with the diagnosis of chordoma.

Conclusion: Although the presenting symptoms and site of tumor are not typical for classical chordoma, cytomorphological features of fine needle aspiration cytology, with aid of immunohistochemical studies, can help establish the diagnosis of chordoma.

Disclosure of Interest: None declared.

P-220

Organizing Venous Thrombus in Superficial Soft Tissue of Lower Leg Aspiration-Biopsy-Cytologically Resembling a Benign Soft Tissue Tumor and Tumor-Like Lesion

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Objectives: We report a single case of organizing venous thrombus in superficial soft tissue of right lower leg initially being suspicious of benign soft tissue tumor and tumor-like lesion, from cytological findings of aspiration biopsy cytology specimens.

Case: A man of mid-forties was aware of a subcutaneous nodule with pain in right lower leg, and admitted to Dermatology Department of our four months later. At the time of admission, the surface of flexure side of right lower leg was slightly elevated in 1 cm in diameter, and bluish in color, and not movable. There was no past history of trauma at this site. A possibility of soft tissue tumor and tumor-like lesion was considered, so aspiration biopsy cytology was performed.

Aspiration Biopsy Cytology: Specimen is composed mostly of blood. A few scattered polyhedral cells were obtained. The few polyhedral cells were non-epithelial and lack of differentiation toward Schwann cells, muscle cells, and vascular endothelial cells. So, we could not make a final diagnosis by an aspiration biopsy cytology specimen.

Macroscopic/Histopathological Findings and Final Diagnosis: A complete excision of the lesion was performed, in order to make a final diagnosis and treatment. The lesion was located within subcutaneous fat tissue and well-circumscribed and red in color. Histopathological findings indicated the lesion was a dilated vein containing an organizing thrombus being full of a vascular lumen. Immunohistochemical study using antibodies to vimentin, alphaSMA, CD34, CD68, and S-100 revealed a presence of active fibroblasts, macrophages, and vascular endothelial cells, and lack of Schwann cells.

Correlation bewteen Aspiration Cytology and Histopathology: The few scattered cells aspirated were indicative of active fibroblasts, macrophages, and vascular endothelial cells from granulation tissue with organization in the venous lumen.

Conclusion: In patients with a clinically superficial blue lesion in extremities, if bloody specimens with a few non-epithelial cells are obtained in an aspiration biopsy cytology specimen, a possibility of organizing venous thrombus should be considered. In a field of pathology of soft tissue tumor and tumor-like lesions, aspiration biopsy cytology is not widely accepted. Aspiration biopsy cytology is less invasive than incisional and excisional biopsy, so aspiration biopsy cytology is a suitable tool for diagnosis of benign soft tissue lesions. Such cases must be accumulated by collaboration with dermatologists, orthopedic and plastic surgeons.

Disclosure of Interest: None declared.

P-221

Benzo(a)pyrene Induce the Inflammation, Nitrosative Stress and Matrix Degradation in Articular Cartilage

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Smoking was associated with increased cartilage loss and persistence of bone marrow lesions in image studies. However, several cohort studies showed smoking as a protective factor for total knee replacement indicated for severe knee osteoarthritis (OA). The association between smoking and risk of knee OA remained conflicting and inconsistent. We would like to use molecular studies for the effects of cigarette smoke on the articular cartilage. Cigarette smoke contains many polycyclic aromatic hydrocarbons, and since dioxins via the aryl hydrocarbon receptor (AHR). Benzo(a)pyrene (BaP), a major component of cigarette smoke, is activate AHR signal pathway. The present study aimed to investigate whether Bap induced inflammation and degradation in articular cartilage in vitro and ex vivo. Primary cultures of chondrocyte, from knee joint obtained at total knee replacement of patients with osteoarthritis, were treated with Bap at a concentration of 0.1, 1, 10 μM for 24 hours respectively. Total cell lysates were collected for western blotting to analyze the inflammatory and catabolic molecules. Bap stimulated chondrocytes overexpression of AHR, cyclooxygenase 2 (COX-2), nitric oxide synthase 2 (NOS2) and matrix metallopeptidase 13 (MMP-13) in a dose-dependent man-

ner by western blotting, as well as MMP-2 by zymography. Bap increased the GAG degradation in extracellular matrix in ex vivo by Safranin-O-Fast green stain. Bap attenuated the type 2 collagen synthesis by Western blotting. Bap activated ERK1/2, AKT signaling in chondrocytes. Consequently, this study demonstrated that Bap induced inflammation, nitrosative stress and degradative enzymes as well as decreased type 2 collagen formation in human chondrocytes. Smoking may be associated with increased cartilage loss without protective effects.

Disclosure of Interest: None declared.

P-222

Primary Cutaneous Cryptococcosis a Cytological and Histological Diagnosis

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We describe a case of a primary cutaneous cryptococcosis (PCC) in an immunocompetent man, diagnosed by Fine Needle Aspiration (FNA) and histological examination of the excised cryuptococcoma, confirmed by special stains and microbiological culture.

A previously healthy 23 year old man with no history of immunosuppression or local trauma, presented with a 14 cm solitary, painful subcutaneous mass in the thigh. CT showed a well circumscribed low density mass in the subcutaneous tissue abutting but not infiltrating the deep muscles. FNA smears revealed pure yeast forms of Cryptococcus surrounded by clear halos. Macroscopically, the excised lesion was a circumscribed subcutaneous mass with a myxoid, gelatinous appearance. Histological examination showed numerous yeasty forms of Cryptococcus with minimal inflammation. Cryptococcus neoformans was confirmed subsequently by microbiological studies. There was no evidence of disseminated cryptococcosis.

Primary cutaneous cryptococcosis can occur in immunocompetent hosts, and is regarded as a distinct entity which carries a much better prognosis that secondary skin involvement by disseminated cryptococcosis. Localised cryprococcosis may present as a solitary mass with gelatinous contents mimicking myxoma. This case also emphasises the role of FNA as a useful modality in the early diagnosis of cutaneous cryptococcosis, especially in clinically unsuspected cases.

Disclosure of Interest: None declared.

Molecular Testing in Non-Gynecological Cytology

P-223

EGFR Mutation Diagnosis with Cytology Specimens from Lung in Non-Small Cell Lung Cancer

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Objectives: Many cases of hospitalized patients with lung cancer can not have to take histologic specimens for diagnosis of Definited Diagnosis & EGFR Mutations. Also, some cases of histological specimens too little those have taken unable to perform diagnostic EGFR mutations. So Lung Cytology Specimens have been used to diagnose EGFR mutations.

Materials and Methods:

Methods: prospective, descriptive with clinical series cases

Materials: Lung Cytology Specimens:

– Bronchoscopy: bronchial washing, BAL fluids, bronchial brushing, TBNA.
– Body fluids: pleural fluids, pericardium fluids, peritonium fluids, cebreral fluids.
– FNAC: lymph nodes FNA, lung tumors FNA, FNA of metastatic tumors etc.
– Sputum.

The Diagnostic Techniques:

– Body Fluids:
* Centrifuge + cyto-smear on glass lame
* Centrifugal casting and cell mass in paraffine (Cell-Block)
* Centrifugal and Cell Deposition (Cytospin Technique)
– FNAC: cyto-smear directly onto the glass lame
– Sputum:
* If there are many malignant cells: cyto-smear directly onto the glass lame
* If there are fewer malignant cells: mucolytic sputum, filtered and condensed cells with Cytospin Technique.

Results:

General data:

– Total cases: **1073 cases** (from September, 2013 to September, 2015)
– Gender: Male: **595 cases (55.45%)**, Female: **478 cases (44.55%)**
– Average age: **58.21 ± 9.56** ages
– The rate of detection of EGFR mutations in common: 448 cases/1073 cases = **41.75%**

The type of specimen to perform diagnosing EGFR mutations at PNT Hospital:

§ Bronchoscopy: bronchial biopsy, transbronchial biopsy, TBNA biopsy
§ Lung Biopsy by Guided CT-Scan
§ Lung biopsy surgery: VATs, Opened Lung
§ Biopsy for metastatic lesions: lymph nodes, soft tissues, skin ect
§ Pleural Biopsy: with Abrams or Castelain Needles, Endoscopic pleura

§ Cytology Samples: bronchial washing, BAL fluids, TBNA, bronchial brushing, FNAC, pleural fluids, sputum etc.

– **The statements:**

§ Particular mutation rate of pathological group (Bronchial Biopsy, Lung Biopsy with Guided CT-Scan, Lung Surgery, Pleural Biopsy and Cytology) have equivalent value (P = 0.057 to 0.089 > 0.05).

§ The rate of mutations in cytology samples have been equivalent value of histological specimens of other group samples and bigger specimens in metastatic tumors.

§ Invalid rate equivalent to other specimens: the rates are from 0.45 to 0.79.

§ Using most types of cytology lung specimens: Fluids in bronchoscopy, body fluids, FNAC, Sputum.

Conclusions: Cytology Lung Specimens of which are used in the diagnosis of EGFR mutation with RealTime PCR. The rate of detection of EGFR mutations in cytology samples have been equivalent histological specimens.

Disclosure of Interest: None declared.

P-224

Detection of EGFR Mutation Status in Cytological and Surgical Pathology Specimens

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Objectives: Epidermal growth factor receptor (EGFR) is a transmembrane protein with cytoplasmic kinase activity that transduces important growth factor signaling from the extracellular milieu to the cell. The EGFR mutations are highly associated with the response to EGFR-targeting tyrosine kinase (TK) inhibitors in advanced-stage adenocarcinomas. In patients with advanced-stage adenocarcinoma, the detection of EGFR mutation status is often invalid due to limited biopsy or surgical specimen, and therefore, cytologic specimens provide an alternative source for molecular testing.

Materials and Methods: In this study, we reported EGFR mutation results from 1261 biopsy or surgical specimens (SP) and 91 cytologic specimens (CP) from 2010 to 2015. In total, we used direct-sequencing and real-time PCR to detect the status of EGFR mutation.

Result: Of the 1261 SP and the 91 CP, 668 (52.9%) and 52 (57%) were positive for EGFR mutation respectively. There is no significant difference between mutation rate and the source of specimen (P = 0.928). Additionally, 7.69% (4/52) of mutation positive specimens had lower tumor content (<10%).

Conclusion: Our data show that cytologic specimens are suitable for detection of EGFR mutation status.

Disclosure of Interest: None declared.

P-225

Prevalence of HPV Types and Its Possible Association with Pre-Neoplastic Anal Lesions Seen in Anal Cytology in Women with History of Squamous Intraepitelial Lesions of the Female Genital Tract in Bogota, Colombia

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Objective: Determine the prevalence of HPV types and their possible association with anal intraepithelial lesions in women with a history of Cervical Squamous Intraepithelial Lesions.

Materials and Methods: A descriptive study, with 131 patients to whom liquid base anal cytology was performed (ThinPrep and SurePath), the age of patients was between 18 and 68 years, and they had history of squamous intraepithelial lesions of the lower genital tract. After processing and diagnosing the liquid base anal cytology samples, typification for the Human Papilloma Virus was made by the technique of linear array ROCHE™ allowing identification of 37 genotypes.

Results: In all patients, anal liquid base cytology was made, the results showed: Negative 121, Positive 9 (5 ASCUS and 4 Low Grade Intraepitelial Squamous Lesion) and 1 Unsatisfactory. The results for the typification, show a surprisingly positive of 67.9%. The most common virus types found were types 6, 16 y 58 which is consistent with previous studies in other populations. In 57.89% of the infections, multiple virus types were present, in 42.11% there was only one type present. 32.9% was a HRHPV infection, 27.6% was Low risk, and 39.5% both HR and LR infections.

Conclusions: HPV testing combined with liquid based cytology are an excellent tool to identify neoplastic and preneoplastic anal lesions in order to allow an earlier clinical forwarding. The presence of HRHPV in anal canal in high percentage must alert and conduct to establish needed guides for screening this type of anal pathology, mostly found in women.

Disclosure of Interest: None declared.

Others

P-226

microRNA-106b Promotes Cell Migration by Targeting DAB2 in Cervical Carcinoma

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Objective: The role of miR-106b and its target gene DAB2 (disabled-2) on the migration of cervical cancer cells was explored.

Methods: The mRNA expression of miR-106b and DAB2 in cervical samples was detected using real time quantitative PCR. The protein expression of DAB2 was examined by Western blot. Dual luciferase reporter assay was used to identification of DAB2 as a miR-106b-directed target gene. Scratch and transwell assay were used to determine the effects of miR-106b and DAB2 on the migration of Hela cells.

Results: The expression level of miR-106b was clearly up-regulated in cervical cancer tissues. On the contrary, DAB2 expression was decreased in cervical cancer specimens. Dual luciferase reporter assay showed that the relative luciferase activity of WT-DAB2-3'UTR decreased approximately 30% after overexpression of miR-106b in HEK293T cells, the results of Mut-DAB2-3'UTR had no difference compared with the control group. DAB2 was identified as a miR-106b-directed target gene. Overexpression of miR-106b in Hela cells significantly promoted cell migration compared with the control group ($P < 0.05$). However, inhibition of DAB2 with siRNA, the rate of migration was increased remarkably ($P < 0.05$).

Conclusion: miR-106b promotes the migration of cervical cancer cells by directly targeting DAB2. These data suggested that miR-106b and DAB2 could play an important role in the pathogenesis of cervical carcinoma, and miR-106b may be as a candidate of biomarker and a potential therapeutic target in cervical cancer.

Disclosure of Interest: None declared.

P-227

Study of the Cigarette Smoking Condensates Effects on Microrna Regulation in Bladder Carcinogenesis and Progression

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Introduction: Bladder cancer is occupies the highest morbidity and mortality of the urinary tract cancer. Urothelial cell carcinoma (UCC) is the most histopathological subtype of bladder cancer. Epidemiological studies have indicated that cigarette smoking doubles bladder cancer risk relative to never smoking, and that current cigarette smoking triples bladder cancer risk relative to never smoking. Cigarette smoking after bladder cancer diagnosis decreases cancer therapeutic response, increases second cancer risk, and worsens post-operative prognosis among cancer survivors. It is necessary to investigate cigarette smoking inductive mechanisms during bladder carcinogenesis and progression. Therefore, administration of cigarette smoking condensate (CSC) in cell experimental model should be useful to realize overall inductive mechanisms of cigarette smoking. For this reason, we designed this study to evaluate the CSC effects on microRNA (miRNA) regulation during bladder carcinogenesis and progression.

Objectives: This study is purposed to elucidate the biological effects and miRNA regulations of CSC treatment during bladder carcinogenesis or progression. Long-term (6 months) treatment of CSC for SV-HUC-1 normal urothelial cell and T24 UCC cell were performed to compare the longitudinal effects of CSC treatment on cell viability, motility and regulations of diagnostic or prognostic miRNAs.

Materials and Methods: Human UCC cell line T24 and the immortalized normal proximal tubule epithelial cell line SV-HUC-1 were employed for analyzing biological effects and molecular regulation of CSC. T24 and SV-HUC-1 are continuously exposed to 0.1 μg/ml, 1 μg/ml, 4 μg/ml (0.1% CSC), and 10 μg/ml CSC in 0.1% DMSO for more than six months. In biological effects, CSC inductive effect on cell viability was evaluated by MTT assay and non-adhersive assay. And CSC inductive effects on cell migration and invasion were evaluated using wound-healing assay, boyden chamber transwell assay, and Matrigel-coated transwell assays. In molecular regulation, CSC modified miRNA expression profile was evaluated by quantitative real-time RT-PCR. Exvivo study used IHC stain to evaluate p-glycoprotein degrade situation.

Results and Conclusion: In this study, we found that CSC remarkably promotes cell viability, migration, invasion and in vitro tumorigenicity in SV-HUC-1 urothelial cells. And CSC also enhances aggressiveness of T24 UCC cells by inducing higher cell viability and motility. In summary, these results indicated that CSC may aggravate bladder carcinogenetic and progressive properties via dysregulation of bladder cancer associated miRNAs.

Disclosure of Interest: None declared.

P-228

The Anti-Proliferative Effect of the Synthesized Diarylheptanoid on Human Prostate Cancer Cells

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Background: Members of zingiberaceae, the ginger family, plants have been used for centuries in traditional medicine. It has received scientific interest as dietary anti-inflammatory and anticancer agents, since it contains curcumin, the principle curcuminoid found in turmeric. *Pleuranthodium racemigerum* (PR) is a tropical Zingiberaceae species plant, mainly in northeastern Australia. Previous studies showed that ethanol extracts of PR is structurally similar to curcumin that contains diarylheptanoid, and also inhibit cancer cell proliferation. Recently, we success-

fully synthesized the diarylheptanoid [1(4'-methoxyphenyl)-7-(4'-hydroxyphenyl)-(E)-hept-2-ene].

Objectives: However, the molecular mechanism of this compound on cancer cell survival remain unclear.

Materials and Methods: Rwpe-1, LNCaP, and PC-3 cells were used in the present study to represent the normal prostate epithelial cells, androgen-dependent prostate cancer cells, and androgen-independent prostate cancer cells, respectively.

Results: Our results showed that the synthesized diarylheptanoid significantly suppresses PC-3 and LNCaP cell survival via the concentration-dependent manners. Additionally, the caspase-3 activity is also elevated upon synthesized diarylheptanoid stimulation in PC-3 and LNCaP cells, indicating that it induces apoptosis in human prostate cancer cells. Furthermore, the synthesized diarylheptanoid also upregulates the p53 protein expression, suggesting that its anti-survival effect is p53-dependent. Meanwhile, we also showed that synthesized diarylheptanoid increased Beclin-1 and LC-3II expressions in LNCaP and PC-3 cells.

Conclusion: In summary, the synthesized diarylheptanoid induces caspase-3-dependent apoptosis and also modulates autophagy in human prostate cancer cells.

Disclosure of Interest: None declared.

P-229

The Acetone Extract of Angelica Sinensis Attenuates Human Prostate Cancer Cell Survival Through Manipulation of Apoptosis and Autophagy Signaling

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Background: *Angelica sinensis* (AS), a common perennial herb in Chinese society, possesses several physiological functions, including anti-anoxia, immune-regulating, anticancer, antibacterial and anti-arteriosclerosis activities. The root parts of AS, rich in volatile oil, vitamins, organic acids, microelements and other organic constituents, has been used as a Chinese medicine for a long time. Human prostate cancer (PC) cells, a common cancer in adult man related to androgen regulation, and it is a second leading cause of cancers death in western countries. Previous studies have shown that acetone extract of AS inhibits a human lung carcinoma A549 cell proliferation via attenuating cell cycle and inducing caspase-dependent apoptosis.

Objectives: However, its effects on PC cells remain unclear, especially on androgen receptor regulation.

Materials and Methods: AS was extracted by different solvents from non-polar to polar (acetone, methanol, to water extraction, AS-a, AS-m, AS-w, respectively), or vice versa (AS-w', AS-m', AS-a', respectively).

Results: The AS-a showed the best antiproliferative effect on PC-3 and LNCaP cells, the androgen-independent and androgen-dependent PC cells, respectively. Additionally, the anti-proliferative effect of AS was better on LNCaP cells than those on PC-3 cells, suggesting that AS-a may attenuate the androgen receptor signaling. Furthermore, AS-a also activates caspase-9 and caspase-3, but not caspase-8, indicating that AS-a induce the mitochondria-dependent apoptosis in LNCaP cells. Meanwhile, we also showed that AS-a upregulates beclin-1, Atg12 and LC-3II expression in LNCaP cells.

Conclusion: Taken together, our results showed that the acetone extracts of AS inhibit prostate cancer cell survival by inducing autophagy and apoptosis.

Disclosure of Interest: None declared.

P-230

Sphingosine 1-Phosphate Inhibits Metastasis by Up-Regulation of TIMP3 Expression Through Suppressing MIR-101 in Chondrosarcoma Cells

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Background: Chondrosarcoma is a malignant tumor that produces cartilage matrix. The most lethal aspect is its metastatic property. Sphingosine one phosphate could affect cell proliferation, inflammation, migration and release of cytokines in serval cells, including chondrocytes in that it promotes osteoarthritis formation.

Objectives: However, its role on human chondrosarcoma is largely unknown.

Materials and Methods: Cell migration ability were determined by boyden chambers. Expression of TIMP-three, Src, MEK, and ERK were analyzed by qRT-PCR and western blot in chondrosarcoma cells.

Results: Here we found that Sphingosine one phosphate increases tissue inhibitor of metalloproteinase three expression and subsequently inhibited cell migration in JJ and SW cells, the chondrosarcoma cell lines. Co-transfection with microRNA one-zero-one mimic reversed Sphingosine one phosphate inhibited cell migration and increased TIMP-three expression. Additionally, pretreatment of cells with c-Src, MEK, and ERK inhibitors or transfection with their specific siRNAs also reversed the Sphingosine one phosphate's effects on cell migration and TIMP-three expression in chondrosarcoma cells.

Conclusion: Taken together, our results indicate that Sphingosine one phosphate inhibited chondrosarcoma metastasis through up-regulation of TIMP-three expression by activation of the c-Src, MEK, and ERK signaling pathway. All of data suggest that Sphingosine one phosphate may be a novel molecular therapeutic for chondrosarcoma metastasis.

Disclosure of Interest: None declared.

P-231

Osteopontin Induces Interleukine Seventeen Production to Enhance Monocyte Migration in Human Osteoblast Like Cells

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Objective: Accumulating evidence indicates that subchondral bone might play an essential role in rheumatoid arthritis (RA). Osteopontin (OPN) induces the production of an important proinflammatory cytokine involved in the pathogenesis of RA. The proinflammatory cytokine interleukin seventeen has been shown to be involved in all stages of the diseaseand to be an important contributor of RA chronicity. However, the signaling mechanism through OPN-induced interleukin seventeen production to influence RA pathogenesis is not clear.

Material and Methods: The expression of OPN was verified by quantitative real-time polymerase chain reaction (qPCR), and Western blot in osteoblast like cells. OPN mechanisms in signaling pathways by Western blot. The monocyte chemoattractant was analyzed by Transwell assays.

Results: Our results showed that directly stimulated osteoblast like cells with OPN increased interleukin seventeen expression in concentration and time dependent manner. OPN induced interleukin seventeen expression mediated by activation of Syk, phosphatidylinositide three kinase and Akt signaling pathways. In vitro chemotaxis assay showed that supernatants from OPN treated osteoblast like cells increased migration of monocyte. In addition, OPN-mediated migration was inhibited by the Syk, phosphatidylinositide three kinase and Akt inhibitors.

Conclusion: Taken together, our results indicated that OPN enhances the migration of monocyte cells by increasing interleukin seventeen expression through the Syk, phosphatidylinositide three kinase and Akt signal transduction pathway in osteoblast like cells. Therefore, OPN may be a potential therapeutic target for RA.

Disclosure of Interest: None declared.

P-232

IFN-Gama Induces Senescence Like Characteristics in Mouse Bone Marrow Mesenchymal Stem Cells

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Objectives: To investigate the effects of IFN-gama on senescence associate properties of mesenchymal stem cells (MSC).

Material and Methods: MSC used in our study were isolated from bone marrow (BM) of mouse. Cell vitalities were measured by CCK8. Phenotypes and ROS of mBM-MSC were analyzed by flow cytometry. Cellular senescence was detected by SA-β-gal stains. IL-6 and CXCL1 secretions were measured by ELISA.

Results: mBM-MSC can differentiated into osteocytes and adipocytes. They expressed CD29, CD106, and Sca-1, while do not express CD31, CD45 and FLK1. Our study showed that cell vitalities of mBM-MSC were siginificantly reduced after IFN-gama treatment for 5 days, and the cell numbers were obviously less after IFN-gama treatment for 5, 10 or 15 days. IFN-gama group increase SA-β-gal-positive cells and reactive oxygen species (ROS) siginificantly after 15 days' IFN-gama treatment. Moreover, IL-6 and CXCL1 secretions were up regulated by IFN-gama.

Conclusions: Our study shows IFN-gama can induce senescence like characteristics of mBM-MSC, suggesting a novel targets for anti-aging therapy.

Disclosure of Interest: None declared.

P-233

The 95% Ethanolic Extract from Typha Angustifolia Has an Anti-Smooth Muscle Cell Proliferation and Anti-Inflammation Effect

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Objective: Atherosclerosis-induced heart failure and stroke are the leading cause of death word wide. Stent implantation is an effective and safe treatment of atherosclerosis. However, about 20–60% of patients will develop a restenosis about 3–6 months after surgery. Neointimal hyperplasia of smooth muscle cells (SMC) is the main cause of the narrowing of artery. The pollen grains of *Typha angustifolia* (TA), a traditional Chinese medicine, has been reported to have a cholesterol-lowing and antiantherogenic effects. In addition to fresh usage, parched TA is used to treat dysmenorrhea, so that TA could be uses as a food supplement to prevent atherosclerosis and reduce the restenosis rate. In this study, the bioactivity of fresh and parched TA extracts were compared.

Materials and Methods: Aqueous and 95% ethanolic extracts of fresh (named as FA and FE, respectively) and patched (named as PA and PE, respectively) pollen grains of TA were used in this research. The polyphenols and flavonoid contents, anti-oxidation, anti-SMC (A7r5 cell line) proliferation, anti-inflammation effects of these extracts were studied.

Result: All of the four extracts contained rich polyphenol compounds (around 34.8–63.0 mg/g). Only FE contained higher flavonoids (30.3+4.5 mg/g), but the flavonoids content of the other three extracts is relatively low. The FE fraction also had a highest DPPH-depletion activity. Activity of 1 mg/ml of FE surpassed that of the control compound BHT. The MTT assay showed that a low concentration (<0.1 mg/ml) of the four extracts was not toxic to A7r5 cells and the macrophage RAW264.7. Among the four extracts, FE can impeded the wound healing of A7r5 cells. The recovery ratio was 47% when treated with 0.1 mg/ml of FE, in comparison to 59% of the control, suggesting that FE may reduce the proliferation and migration of SMC. Zymograph study showed that matrix metallopeptidase 9 (MMP9) production was reduced to 75% in comparison to control by 0.2 mg/ml of FE. FE can dose-

dependently reduced the LPS-induced NO production in cell line RAW264.7.

Conclusion: The data demonstrated that the ethanolic extract has a best anti-SMC proliferation and anti-inflammation effect. It could be used as a food supplement to prevent cardiovascular disease. Since the FE fraction contained the highest flavonoids, which could the main bioactive compounds in the FE fraction.

Disclosure of Interest: None declared.

P-234

The Transition Zone Stability and Ciliary Protein Homeostasis of Primary Cilia are Maintained by TCTN2

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The transition zone (TZ) serves as a diffusion barrier to regulate the ins and outs of the proteins recruited to the primary cilia. TCTN2 is one of the TZ proteins and its mutation causes Joubert syndrome, a serious multi-organ disease. Despite its important medical relevance, the functions of TCTN2 remain elusive. Here we created a TCTN2 gene deleted retinal pigment epithelial cells (RPE1) using CRISPR/Cas9-based genome editing technique and used this knockout line to reveal roles of TCTN2. TCTN2 knockout RPE1 cells displayed a significantly reduced ciliogenesis or a shortened primary cilium length in the cilium-remaining population. Intraflagellar transport protein IFT88 aberrantly accumulated at the tip of TCTN2 deficient cells. Guanine nucleotide exchange factor Arl13B was mostly absent from the ciliary compartment, with a small population localizing at the ciliary tip. The deficient TZ was corroborated with the mislocalization of two other TZ proteins TMEM67 and MKS1. In addition, TCTN2 deficiency induced TZ impairment led to suppression of Sonic hedgehog signaling in response to Smoothened (Smo) agonist. Together, depletion of TCTN2 destabilizes other TZ proteins and considerably alters the localization of key transport and signaling-associated proteins, including IFT88, Arl13B, and Smo.

Disclosure of Interest: None declared.

P-235

Study of Interactions and Tissue Tropisms of Free-Living Amoeba with/To Human Host Cells

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Objectives: Free-living amoeba can enter host body through the nasal olfactory passage and haematogenous route, and trigger inflammatory response at the side of it infected such as brain and eye. However, there is no better study on the pathogenesis of the inflammatory response. The interaction, tissue tropism and pathgenesis of induced inflammation (especially of COX-2 reaction) between free-living amoeba and host cell need to be further investigation.

Materials and Methods: In this study we analyzed the role of the TLR and its signaling pathway in human microvascular endothelial cells (HMECs) challenged by Acanthamoeba.

Results and Conclusion: The study relvealed that the mRNA expressions of TLR4, myeloid differentiation protein 88 (MyD88), nuclear factor (NF)-kB, Cyclooxygenase 2 (COX-2), and the inflammatory cytokines interleukin (IL)-8, and interferon (IFN)-β were significantly increased in Acanthamoeba-treated cells. TLR4 is a receptor for Acanthamoeba and exerts an effect through TLR4-MyD88-NF-kB pathways to induce the secretion of cytokines in HMEC under *Acanthamoeba* challenged and also exerts some factor to turn off the pathways and inflammatory responses. It needs more research and study to understand how and what about the interaction between *Acanthamoeba* and host cells.

Disclosure of Interest: None declared.

P-236

The Effect of Retinoic Acid in the Migratory Phenomenon of Acral Melanoma Cells

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Objectives: Acral melanoma (AM) is a notorious skin tumor in non-White population. However, the pathogenesis of AM is still ambiguous. Retinoic acid (RA) signaling is essential for melanocyte differentiation and homeostasis.

Materials and Methods: We conducted a hospital-based retrospective review of ALDH1A1, ALDH1A3, CRBP1, and CRABP2 distribution immunohistochemically in samples of Acral melanoma. ALDH1A1, ALDH1A3, CRBP1, and CRABP2 immunoreactivity was in AM and try to correlate with the classic' parallel ridge pattern'.

Results: In this study, we first demonstrated that endogenous RA was abundant around acrosyringium of eccrine duct. Then we confirmed that variously accentuated expressions of the four RA-related proteins in the same region of distal eccrine duct. Although some AM cells showed higher ALDH1A1, most of them revealed lower expression of ALDH1A3, CRBP1 and CRABP2.

Conclusion: Our results indicated that RA might contribute to the migratory phenomenon of AM cells. We provided new information that RA could drive the chemotactic migration during AM pathogenesis. This discovery implies that the RA effect could be one of therapeutic directions in AM.

Disclosure of Interest: None declared.

P-237

Report on the Phylogenetic Studies of Diplostomatids Parasites Collected in Freshwater Fish

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Introduction: Diplostomid metacercariae inhabit freshwater fish species as the second intermediate hosts. These parasites have been found in the eye lens, the retina, vitreous humor and the nervous system of freshwater fish. The classification of these parasitic stages to the species level using only morphology is often difficult and ambiguous. The use of molecular techniques has allowed links to be elucidated using various developmental stages of these parasites. The aim of this study was to provide a summative report on the phylogenetic tree by applying molecular biology techniques to the investigation of larval diplostomid parasites.

Materials and Methods: Diplostomid metacercariae were preserved in 70% ethanol prior to DNA extractions using Qiagen kit. Standard techniques for amplification of rRNA region were followed. The DNA amplicons were sent to inqaba Biotech laboratory for sequencing and phylogenetic trees generated using software programs.

Results: The amplicons of these diplostomids had band sizes of 500 base pairs. The amplicons contained only partial regions (ITS-2). The parasitic species 28S rDNA genomic region was successfully amplified.

Conclusion: The application of molecular techniques on digenetic trematodes seems very promising and may yield great potential in future descriptions of morphologically similar parasitic species.

Disclosure of Interest: None declared.

P-238

Whole Slide Imaging for Digital Cytopathology in Big Data Era

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Objective: Digital pathology has become the key turning point in the development of pathology, which based on the combination of computer and the internet. However, little is known on the digital cytopathology. The present study tries to explore the application of whole slide imaging for digital cytopathology in the big data era.

Material and Methods: The data in the hospital has become one of the most important sources during the progression of digital medicine, including the case history, image and telemedicine. 60 cases of thyroid fine needle aspiration slides from 6 different pathology departments were scanned using Hamamatsu NanoZoomer-XR operated by 6 different operators. Remote eveluation and case study were applied for 30 cytopathologists from 10 different provincial hospitals in China. The factors affecting the quality of the digital cytopathology were compared.

Results: This study suggested that slide scanning technology is the most important technology of digital pathology, as well as digital cytopathology. However, digital cytopathology progressed slowly comparing with the digital pathology. Those factors affect the quality of the digital cytopathology including: cell smear quality, staining quality as well as the skill of the operator, image quality, speed of the internet and security of the data. And cell smear quality and staining quality are the key points of the quality of the digital cytopathology.

Conclusion: Whole slide imaging for digital cytopathology could be applied for the remote consultation and online education, which is the most promising way for the progression of cytopathology diagnosis in the Big Data Era.

Disclosure of Interest: None declared.

P-239

The Role of Whole Slide Imaging in the Diagnosis of Non-Gynecological Cytopathology

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Introduction: Whole slide imaging (WSI) is an emerging tool in the research application and clinical practice of digital pathology. This WSI systems have the potential to become useful tools in cytopathology practice. This study aims to investigate the accuracy and concordant rate of WSI when compared to the standard diagnosis by glass slide in cytopathology of nongynecological sections.

Material and Methods: Ten non-gynecological cytology slides were selected and qualified by two pathologists. All of them were scanned by Aperio Scan Scope slide scanner. The multiple focal

plan capture function with 40x scan was performed. All specimens were interpreted by an experienced and WSI-trained pathologist and compared to the standard glass slide with optical microscope. The time between the interpretation of WSI and glass slide were three weeks. They were analyzed in two aspects: First, the accuracy rate of diagnosis; and second, the concordant rate categorized as either an identical, minor, or major difference.

Results: All ten WSI were justified as satisfactory capture. The accuracy rate of WSI was 90%. For WSI, sensitivity was 91.67% while the specificity was 100%. The PPV of WSI was 100%; NPV was 94.74%. Kappa coefficient was 100% (p value <0.001); concordant rate was 90%; and the disconcordant rate was 10%. The major difference was seen in 1 case, which was papillary carcinoma of thyroid gland in a FNA specimen. Which the diagnosis of WSI was AUS but the diagnosis of standard glass slide was papillary carcinoma. The mean of interpretation time of WSI and standard glass slide are 4.56 and 2.11 minute, respectively.

Discussion: This study show better concordant rate of WSI compared to previous study (90% and 75%, respectively). The multiple focal plan capture function may solve problem of multilayer of cytopathology. This study show potential of WSI in cytopathology. But there are any disadvantage (unclear nuclear detail and longer interpretation time) of WSI. The longer interpretation time may due to the necessary use of a computer mouse to pan the image over a monitor made it uncomfortable to use WSI. We suggest that the new version of WSI should be made much easier using currently available WSI viewer tools for easy and systematic navigation of WSI (such as thumbnails, autopanning, keyboard navigation, and tracker capabilities). The author suggest that the WSI should be suitable for learning objective in laboratory demonstration but should not be use in routine service.

Conclusion: WSI has the potential to play a major role in the diagnosis in non-gynecological cytology but at the present time, it does not show any advantage over glass slides and optical microscope. In the future, a higher-resolution scan of WSI could be worked out to address technological limitations.

Disclosure of Interest: None declared.

P-240

MU-TNED: An Electronic Database Focused on Long-Term Study of Thyroid Nodule

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Objectives: The University of Missouri Thyroid Nodule Electronic Database (MU-TNED) was designed to focus on patients with thyroid nodules and allow organization and management of data for:

1) Review of epidemiological and clinical data in thyroid nodules

2) Quality Control and Quality Improvement (QC/QI) of Fine Needle Aspiration (FNA)

3) Long term survival of thyroid cancer patients

4) Impact of emerging molecular diagnostics

5) Collaboration between researchers and clinicians in multiple institutions.

Materials and Methods: Database Design: A multidisciplinary team with a focus on thyroid nodule was created at MU including departments of pathology, endocrinology and health informatics. MU-TNED database was designed with the Research Electronic Data Capture System (REDCap), a Vanderbilt-developed, secure, web-based platform for building and managing research databases. MU supports REDCap through the Institute for Clinical and Translational Science (ICATS). Patient Metadata information: The required metadata information (ex. field name, end-user label, data type, data range, etc.) was used to build and populate the study-specific database tables feeding a working web-based electronic data collection (EDC) application for the project. The team identified patients with specific ICD 9 through an i2b2 search. Corresponding demographic data, pathology, FNA results, and imaging information was imported to initialize the long term electronic data repository.

Result and Conclusions: The MU TNED was created over a period of 3 months. Once the web-based platform was built, it was tested for quality and accuracy of data imported. The web application was successfully used to import 935 thyroid nodule cases from 2008 to 2012. The database contains demographics as well as emerging technologies and therapies. The database was then tested for clinical and QC/QI studies on 1) diagnostic accuracy of FNA, 2) Bethesda System Reporting of Thyroid FNA, both presented at the 63rd American Society of Cytopathology Annual Meeting.

Increasingly, in healthcare, use of informatics supported tools enables providers to improve patient care and collaborate in translational research. MU-TNED was specifically designed to follow patients in a longitudinal manner and support multiple aspects of research. It allows flexibility to expand to store and analyze additional emerging imaging and molecular diagnostics. The data will be readily available for QI studies involving multiple disciplines and centers. Long term follow up of patients and the ability to collaborate is essential because of the nature of this disease; a disease with high prevalence but low mortality, therefore collaborative data analysis may provide more answers, especially related to long term population outcomes. This will allow MU-TNED to establish national and international collaborations.

Disclosure of Interest: None declared.

P-241

Utility of $p16^{INK4a}$/Ki-67 Double Labelling Immunostaining (CINtec® PLUS) for Evaluation of Squamous Lesions in Conventional Smears

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Background: Interpretation of squamous lesions might be problematic due to a variety of factors including improper slide processing, atrophic epithelial patterns and other mimickers for abnormality of squamous cells. Double labelling method for $p16^{INK4a}$ and Ki-67 proteins (CINtec®*PLUS*) have been recently shown to differentiate high grade squamous abnormalities from lower grade lesions. Most studies have performed on liquid based materials under well controlled conditions. This study aim to ex-

plore its utility on conventional smear under uncontrolled conditions.

Materials and Methods: A total of 83 slides with abnormal squamous lesions submitted from outside laboratories for consultation from January 2012 to December 2014 were subjected to this study. These cases, categorized by The Bethesda System 2001, included 9 LSIL, 22 LSIL-H, 8 ASC-H, 41 HSIL and 5 SCC. 4 NILM were also added in the study as negative control. All slides were double ICC stained for $p16^{INK4a}$ and Ki-67 using CINtec®*PLUS* cytology kit with Ventana™ automated stainer. Interpretation of slides were performed using algorithm suggested by company's instruction. A minimum of single cell with concurrent staining for p16 (cytoplasm) and Ki-67 (nucleus) was considered positive results. Only 29 (32.6%) cases with histologic follow-up were available in the study. These included 4 LSIL, 5 LSIL-H, 1 ASC-H, 17 HSIL and 2 SCC. The results were regarded as gold standard for analysis.

Results: Out of 87 slides stained, 31 (35.6%) gave positive results that included 5 (22.7%) LSIL-H, 1 (14.3%) ASC-H, 22 (55.0%) HSIL and 3 (60.0%) SCC cases. None of 4 NILM and 9 LSIL were demonstrated any double labeling. Out of 29 abnormal squamous lesions, 19 (65.5%) cases had histologic follow-up of high grade lesion namely HSIL or higher in which cytological diagnosis of HSIL (88.2%) and SCC (100.0%) gave highest incidence of high grade histologic outcome.

Case-by-case comparison with double labeling results, 12 out 19 cases with HSIL showed positive staining results whereas 7 out of 10 cases with LSIL gave negative results. Thus, sensitivity and specificity of the test based upon histological proved cases was 63.2% and 70.0%, respectively. Positive predictive value of this test is 80.0% whereas negative predictive value is 50.0%.

Conclusion: Double labeling immunostaining for $p16^{INK4a}$ and Ki-67 in slides under random uncontrolled processing conditions had relatively low sensitivity in comparison with liquid based and well controlled settings in the literatures. Nevertheless, high positive predictive value indicated utility of the test in problem cases where positive staining was associated with high grade histologic outcome. Again, negative staining results did not preclude presence of high grade lesions in the follow-up.

Disclosure of Interest: None declared.

P-242

Novel Well-Coagulum Clot Cell Block Method on Fine-Needle Aspiration

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Objective: Conventional formalin-rinsed cell block (CF-CB) preparation is a relevant method for fine-needle aspiration diagnosis of cancer in many organs. However, the limitation of its quantity and quality of cells and time consummation still exists. A novel well-coagulum clot cell block (WCB) is developed to address these limitation.

Material and Methods: Fine needle aspiration materials obtained from various organs including livers and thyroid glands that were performed during January to June 2015 at Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand, were included. Rapid onsite evaluation of cytological smears were performed in each cases to ensure adequacy of the aspirates. Designated passes were dedicated for either CF-CB and WCB methods of cell block preparation. Cell block histologic sections were evaluated for adequacy for cell block preparation, cellular shrinkages, presence of diagnostic materials, and suitability for immunohistochemical stainings.

Results: 12 cases of CF-CB and 12 cases of WCB were obtained during the period of study. 2 cases of CF-CB were excluded due to insufficient materials in both cytological smears and cell block preparations. 1 case of CF-CB obtained from cystic fluid and 1 case of WCB obtain from pus was also omitted due to absence of epithelial components. Thus, 9 CF-CB cases were compared with 11 WCB cases. All material showed adequacy of either cell pellets or clot for cell block preparation. Cellular shrinkage was noted in only in 4 cases of CF-CB (44.44%) whereas none of 11 WCB displayed any shrinkage. All 9 CF-CB cell blocks contained diagnostic materials whereas 10 out of 11 cell blocks from WCB obtained diagnostic samples. Only 2 cases of CF-CB and 3 cases of WCB were processed for immunohistochemical stainings. Both methods displayed comparable antigen preservation and suitability for stainings. Nevertheless, time consummation for WCB was much less than CF-CB due to less steps involved in the process.

Conclusion: A novel well-coagulum clot technique was developed to suppliant conventional methods for cell block preparation for fine needle aspiration material. Adequacy, cellular and antigenic preservation of this method was at least comparable to the conventional methods. The clear advantage is time consummation due to less processing steps.

Disclosure of Interest: None declared.

P-243

NASA Task Load Index for Digital Cytology. Comparative Analysis of Input Devices

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Objectives: Scanning whole slide images (WSIs) of cytology specimens poses several obstacles compared to histopathology slides based mainly on the thickness of the sample. Image control mechanisms for viewing cytology WSIs have not been enough studied from an ergonomic point of view. The aim of this trial was to investigate a proper input device for digital cytology.

Material and Methods: Five medical students from different international universities tested 6 input devices randomly for digital cytology: conventional mouse (HP), Rollermouse (Contour), 2 trackballs (Logitech and Ulove), vertical mouse (CLS), and ErgoPointer (Märzhäuser) in a time-controlled exercise handling six cytospin samples (urine, cerebrospinal and pleural) scanned at 40X with volume scanning (3 Z-stack layers of 2 microns interval) using iScan Coreo (Ventana) and Image Viewer (Ventana). The exercise consisted of identifying the sample and guiding a diagnosis, having previously been explained all general concepts in these kinds of specimens. NASA Task Load Index which includes 6 subscales: mental, physical and temporal demands, performance, effort and frustration, was used to rate the perceived workload for each device separately.

Results: Participants (female = 3, male = 2, age = 23.2, $\sigma = 0.98$) completed the exercise. Average perceived workload/duration (in seconds) of the exercise with each input device were: conventional mouse (324/300s), Rollermouse (462/312s), Ulove trackball (646/341s), Logitech trackball (465/332s), vertical mouse (403/303s), ErgoPointer (881/369s). Statistical positive correlation between the length of the exercise and the perceived workload for each device existed ($p < 0.05$). No correlation existed between the sequence of the devices during the exercise and the perceived workload or length.

Conclusion:

– We describe a systematic and reliable test that permits evaluating input devices among users to find an optimal one for digital cytology.

– The test is designed also for evaluation of important ergonomic issues in order to avoid work-related musculoskeletal disorders.

– Despite ErgoPointer represents a transition between microscope and mouses, students had worse time score and perceived higher workload during the exercise. Regarding to this, pathologists should do this kind of trial to contrast these results.

Disclosure of Interest: None declared.

P-244

Gel-Tube Technique: A Cost Effective and Sample Sparing Method for the Preparation of Compact Cell Blocks

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Objective: The cell block (CB) technique has undergone various modifications over the past few decades. Although CBs are routinely used for diagnostic testing, the variations in CB volume and cellularity remain the major barrier to ensuring sufficient cell quantities.

Herein we introduce a simple and cost effective CB technique suitable for many types of cytology specimens. The new 'gel-tube' (GT) technique leads to compact CBs with constant cell quantities, more sample-sparing compared to traditional CBs.

Material and Methods: A specialized device was developed for the preparation of a cylindrical shaped, compact CB. The GT was made by 3% agarose, with a uniform shape and cross section surface. We applied it to the prefixed cytologic samples to obtain a cell pellet with maximal cell density. In the current study, the GT technique was used to prepare CBs and its efficiency was compared with that of the conventional plasma thrombin clot (PT) method. Six nongynecologic cytology specimens of different origin were collected for CB preparation. H&E sections and immunohistochemical (IHC) study for tumor cells were performed. To validate the applicability for molecular tests, the DNA yield of each sample was analyzed by optical density.

Result: The GT technique provided smaller section area (2–15 mm^2) than the conventional PT method (90–600 mm^2). Increased tumor percentage and homogeneity were proved by serial sections for IHC stains. In addition, the GT-CB specimens could be arrayed in a single slide for the purpose of the molecular panel detection. The concentrations of DNA from GT-CB and PT-CB specimens varied. Briefly, a maximum yield (4.604 mg) was acquired from a GT-CB specimen. Further, the double-stranded DNA wasn't lost from this sample sparing method.

Conclusion: In this preliminary study, we demonstrate the feasibility of generating compact CBs with cellular homogeneity and optimal DNA yields. By using the GT technique, it is possible to have diagnostic cells evenly distributed throughout the different section levels. Besides, the proposed benefit of such a sample-sparing protocol is to enhance tumor cellularity, especially for cytologic samples obtained from limited or residual diagnostic materials. Our new approach can be further applied in detecting novel markers high throughput to minimize the invasive diagnostic procedure. More studies are required to explore the applicability of this new CB technique.

Disclosure of Interest: None declared.

P-245

Nano-Pathology Imaging Using Cytology Materials

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Objective: To decide on courses of treatment, HER2 immunohistochemical staining in breast cancer material has become common. DAB staining is struggling to often determine from facility difference occurs. Nano-path staining using a fluorescent nanoparticle is possible to the quantification of proteins, reduce individual variability of pathologist and facilities, stratification of patient. Since cytology is less invasive, it is possible to determine the direction of the screening phase, we examined whether it is possible to protein quantification using cytology material.

Methods: Human breast cancer SK-BR-3 cell line was fixed in liquid-based cytology fixations (SurePath, ThinPrep, TACAS) and each slide were made with manufacture's procedure. SK-BR-3 were examined by nano-patho staining with HER2, EpCAM, Cytokeratin18, Ki67 antibody and analyzed using dedicated software.

Results: HER2, EpCAM, CK18 was detected in cell membrane and Ki67 could not be confirmed in nuclear expression due to does not penetrate into the nucleus. The difference in expression amount by LBC fixation was confirmed that ThinPrep is the highest bright points, SurePath, TACAS was equivalent of the bright points.

Conclusions: It was suggested that nano-patho method is useful for quantitative protein analysis of cell membrane and cytoplasm. It was confirmed that the nuclear antigen detection is need for membrane penetrating process. The reason for the difference with the fixed solutions occurs, the main component is a difference by either ethanol (SurePath, TACAS) or methanol (ThinPrep). In addition, cytology material is a three-dimensional, bright points may depend on the imaging point, it was considered to require to devise when nano-patho analysis is performed.

Disclosure of Interest: None declared.

P-246

Effective Approach for Collecting Individual Cells and Measuring a Single Cell Adhesion Force

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Single cell analysis have become an essential part of basic and clinical research. Similarly, the investigation of cell adhesion properties is a key issue in substrate mediated cell behavior essential for understanding cellular properties in both health and disease. A prerequisite to these studies is the isolation of individual cells with a follow up measurement of cell adhesion force. There are commercially available single cell collection technologies including laser based systems and cell sorting instruments. There are also approaches for single cell adhesion force measurements including atomic force microscopy, optical tweezers, and micropipette/capillary aspiration. However, none of the existing instruments provide concurrent single cell acquisition and adhesion force measurement prior to its preparation for the downstream analysis.

Objectives: We proposed development of a universal platform for single cell acquisition, measurements of its adhesion strength and deposition into the single wells for further molecular analysis or clonal expansion.

Materials and Methods: Cell acquisition properties of the developed instrument are based on the capillary based vacuum pulse assisted technology (CTAS; Kudo et al, 2012). Developed system is capable of collecting individual cells from any adherent cultures grown in standard cell culture dishes in as small as 15 nl volume, compatible with various downstream single cell analyses and next generation sequencing. Measurement of cell adhesion force is performed based on the capillary aspiration techniques reported earlier. The system utilizes an inverted microscope, so that the cells of interest can be identified based on morphology, location or labeling, including fluorescence.

Results: Individual cells were collected from human neuroblastoma SH-SY5Y, CHO, and 3T3 cell cultures. Collected single cells were dispensed immediately into individual wells for clonal expansion. Clonal expansion for 7 days of these single cells revealed minimal effect on cellular viability (up to 99% when compared to dilution controls). Moreover, trypan blue assay demonstrated survival rates similar with the re-cultivation studies. Cell adhesion force measurements were performed for multiple cells from all three cell lines.

Conclusion: The benefits of the proposed technology include cost-efficiency, simple operation, complete workflow from single cell isolation to adhesion force measurement, compatibility with a wide range of inverted microscopes and use of standard plates and culture dishes. The instrument offers a solution to numerous applications that require single cell acquisition and measurement of a single cell adhesion force. It can be used upstream of various cell and region specific analyses, such as NGS and initial characterization of cell adhesion properties.

Disclosure of Interest: None declared.

P-247

Preparation of High-Quality Cytoscrape Cell Blocks from Conventional FNA Cytology Slides of Lymph Nodes: A Technical Report on a Modified Method

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Background: Immunocytochemistry (ICC) is one of the most commonly used tools for the differential diagnosis of difficult cytology cases. We introduce a modified agarose-based cytoscrape cell block technique that can be effectively used preparations of cytoscrape cell block (CCB) sections for ICC.

Methods: A representative Papanicolaou-stained conventional fine-needle aspiration (FNA) cytology slide from each case was decoverslipped and covered with a small amount of alcohol. A cell scraper was used to scrape the entirety of the cytologic material off the slide and harvested into a tissue mold. The cytoscrape material was pelleted by centrifugation and pre-embedded in ultra-low gelling temperature (ULGT) agarose and then re-embedded in conventional agarose. The final agarose gel disk with the cell button at the bottom was put in a tissue cassette for routine tissue processing for the preparation of a paraffinized CCB.

Result: The quality of the ICC on the CCB sections was comparable or identical to that of the immunohistochemical stains on histologic sections. By scrapping and harvesting the entirety of the diagnostic material off the cytology slide into a compact agarose cell button, we could avoid the risk of losing diagnostic material during the preparation of the CCB.

Conclusion: A high-quality cell block can be can be prepared from the cytoscrape of a conventional FNA cytology slide. The sections of the cytoscrape cell block can be effectively used for *in situ* analysis of the targets in diagnostic cell and tissue obtained by FNA.

Disclosure of Interest: None declared.

P-248

Fine Needle Aspiration Cytology (FNAC) Simulation Using Phantoms. University Teaching Experience

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Objectives: Fine needle aspiration cytology (FNAC) is a minimally invasive and extremely useful procedure with a low-risk of injury traditionally made by pathologists. The characteristics of pathology practices and limited equipment make teaching in this technique difficult, missing the opportunity to attract and recruit future pathologists. We therefore have introduced phantoms designed to perform FNAC in the educational process in our hospital.

Materials and Methods: Phantoms are two life-sized handmade anthropomorphic reproductions of a head & neck and a trunk, respectively, coated by silicone simulating skin with inserted tumor areas (utility model ES1140059). They are inspired by other patents (US6485308, US5803746) and improved, including the whole FNAC process (palpation, puncture, aspiration, expel material on slide, and smear preparation), having human shape and being reusable. They allow performing FNAC, obtaining samples of cream material to be extended on slides. The practice consisted of obtaining an FNAC samples in a clinical context by each student individually, with a subsequent cytological correlation using whole slide imaging.

Results: 116 medical students, in their third year, from the University of Murcia, Spain, took part in the FNAC practice (16 groups: 66 women, 50 men). The success rate in the first attempt (puncture, aspiration of material, expelling and extending the obtained material on slides) was 96.5%. In Addition, 13 students from 10 other universities (national and international) conducted the same practice, referring to not having this opportunity in their places of origin and considering the practice to be valuable in an anonymous survey.

Conclusion: FNAC practices are easily implementable in the undergraduate curricula. There is no proper uniformity or standardization in the practices among different universities. FNAC simulation provides students with greater knowledge and appreciation of our specialty.

Disclosure of Interest: None declared.

P-249

Analysis of 740 Cases with Peritoneal Washing Cytology in Gynecologic Epithelial Cancer

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Objectives: Peritoneal washing cytology has been used widely to detect subclinical intraperitoneal metastases in gynecologic cancers. However, the role of washing cytology as a sensitive indicator of peritoneal extension in gynecologic cancers has long been debated. Though peritoneal washing cytology has been eliminated from the endometrial staging system, the AJCC still recommended that the washing cytology should be reported. This study evaluated the operating characteristics of individual gynecologic epithelial cancer at National Taiwan University Hospital (NTUH).

Materials and Methods: We retrospectively evaluated 799 consecutive patients with primary gynecologic epithelial tumors who had peritoneal histology and concomitant washing cytology performed during initial surgery at NTUH from January 2000 to December 2014.

Result: Fifty nine patients (7.4%, 59/799) with unsatisfactory cytological results for evaluation due to severe degenerative change were excluded, while the remaining 740 patients were analyzed. There were 442 patients with epithelial endometrial carcinomas, 236 with ovarian carcinomas, 46 with borderline ovarian tumors, nine with cervical carcinomas, six with Fallopian tube carcinoma/borderline tumor and one with vagina carcinoma. Peritoneal washing cytology was positive at initial surgery for 133 (18.0%) of 740 patients, including 54 of endometrial carcinomas, 76 of ovarian carcinomas, two of borderline ovarian tumors and one patient of cervix carcinoma. By using peritoneal histology as the gold standard adjusted with clinical follow-up, the operating characteristics of washing cytology in endometrial carcinomas were as follows: sensitivity 75.0%, specificity 94.0%, PPV 55.6%, NPV 97.4%, and accuracy 92.3%; while the characteristics in ovarian carcinomas were listed as follows: sensitivity 55.3%, specificity 85.7%, PPV 75.0%, NPV 71.3%, and accuracy 72.5%.

Conclusion: The sensitivity of peritoneal washing cytology was relatively low in comparison with peritoneal histology in our series. More liberal use of biopsies in suspicious areas, more sophisticated approach in peritoneal washing procedures, and shorter delay in sampling processing to prevent degenerative change may be the keys in quality improvement of future practice.

Disclosure of Interest: None declared.

P-250

Diagnostic Difficulties in Fine-Needle Aspirations from Parathyroid Lesions

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Objective: The aim of this study is to review the preoperative cytologic diagnosis of parathyroid lesions (PLs), to search for diagnostic difficulties and to look for morphological clues for correct interpretation.

Materials and Methods: 364 resection specimens are found in the archives diagnosed as parathyroid adenoma/hyperplasia between 2009–2015; 48 had preoperative fine needle aspirations (FNA) from thyroid/parathyroid region. The localization and size of the aspirated and excised nodules were compared; 14 cases showing entire match were included in the study. Three cases from neck or thyroidectomy bed (TB) in which the parathyroid origin was confirmed by immunohistochemistry were also added.

Results: Ten of 17 cases were clinically suspicious for PLs; in 4 of them cytologic examination and immunohistochemistry (IHC) confirmed the diagnosis; 2 had hypocellular aspirates inadequate for interpretation; 4 cases could not be distinguished from thyroid tissue on FNA either due to misleading results or total lack of IHC. Preliminary clinical diagnosis was thyroid nodule in 4 cases of which one was diagnosed as PL on FNA; the other 3 cases were thought to be thyroid nodules also on FNA and categorized as suspicious for papillary thyroid cancer, follicular neoplasia (FN/SFN) and atypia of undetermined significance (AUS/FLUS), respectively. Three FNAs, 2 from TB and one from neck, without any clinical preliminary diagnosis were interpreted as parathyroid lesions on cytology. Microscopically, most of the cases showed microfollicular pattern; however some were characterized with papillary like structures or small clusters/sheets. Isolated nuclei in the background were common. Vascular network was conspicious in most cases. Cells had round, small to medium sized nuclei with fine granuler chromatin.

Conclusion: Parathyroid tissue could have been correctly identified in only eight of 17 cases, whereas 4 cases remained without a definitive diagnosis and 5 cases were interpreted as thyroid nodules of which 3 found to be atypical or suspicious. Cellular materials with microfollicular/pseudopapillary pattern, rich in vascular network, with many bare nuclei in the background should alert the pathologist for parathyroid tissue, especially if the nodules are localized to the poles of thyroid gland. In FNAs from TBs of patients with a suspicion for recurrent disease, parathyroid tissue should always be considered in the differential diagnosis. Immunohistochemistry or parathormon levels in the needle rinse are needed for definitive diagnosis, so obtaining extra material is necessary from patients whose aspirations suggests parathyroid tissue either clinically or on rapid on-site evaluation.

Disclosure of Interest: None declared.

P-251

Mucinous Balls in Fine Needle Aspiration Cytology Smears of Prostate

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Objectives: To describe the mucinous balls in the aspiration smears of prostatic lesions and to discuss their importance.

Materials and Methods: Fine needle aspiration smears were obtained from enlarged prostates of 69 patients just before the needle biopsy procedure. Four smears were prepared from each case, two were air-dried and two were fixed in90% ethanol and stained with Wright-Giemsa and Papanicolaou methods respectively. The smears were screened independently by three pathologists.

Results: Routine clinical investigations revealed difficulty in urination, sonographic evidence of prostatic enlargement and abnormal prostate specific antigen. Clinically the cases were suspected for malignancy and advised needle biopsy. The smears of 51 cases were diagnosed as benign, but histology sections confirmed benign prostatic hyperplasia in 40 cases only, the other 11 cases were diagnosed as low-grade adenocarcinoma, gleason's score 3+3. The smears revealed hypercellularity composed of isolated cells with low nuclear-cytoplasmic ratio, well defined smooth nuclear border and without nucleolus, epithelial cell clusters with benign looking cells, good number of lymphocytes and rare glandular structures. Interestingly the smears in four cases showed many mucinous balls in the back ground and a few of them attached to epithelial cell clusters. These ball like bodies are large, round and pink in color without onion-peel appearance. They were stained positively with alcian blue. The smears of 18 cases were diagnosed as positive for malignancy, but histology sections were diagnosed them as high-grade adenocarcinoma, gleason's score5+5[16 cases] and as low-grade adenocarecinoma, gleason's score3+3[2 cases]. The smears of these cases revealed hypercellularity composed of epithelial cell clusters and many isolated malignant cells with high nuclear-cytoplasmic ratio, hyper chromatic nuclei with irregular nuclear border and prominent nucleoli. Also mucinous balls were noticed in the background of two cases.

Conclusion: Large round pink mucinous balls without onion-peel appearance were noticed in the background of smears in 6 cases, which were not reported before. They were noticed both in benign and malignant lesions, so they were not helpful to differentiate benign from malignant lesions. They were different from corpora amylacia.

Disclosure of Interest: None declared.

P-252

The Study of the Correlation between Tumor Suppressor FOXO3 and Recurrence Rate in Bladder Cancer

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Bladder cancer, most commonly urothelial cell carcinoma (UCC) is the ninth most common cancer among men in Taiwan. Biomarkers in bladder cancer are grouped according to their molecular type-DNA (hOGG1), RNA (cadherins), or protein (COX-2, mTOR, PTEN, c-myc, p27, phos-Akt, and phosS6, p53, FOXO). FOXO1 and FOXO3 are key regulators of protein breakdown also been implicated in the regulation of the cell cycle, apoptosis, muscle regeneration, oxidative stress resistance, and DNA damage repair. The FOXO3a and FOXM1 are key players in cancer initiation, progression, and drug resistance. Dysregulation of FOXO3a activity and localization has been associated with cancer initiation and progression, as well as chemotherapeutic resistance including pancreatic, acute myeloid leukemia and bladder cancer. In this study, we surveyed the expression of FOXO3a in collected clinical specimens of bladder cancer patients by immunohistochemistry and western blot method. The correlation between the tumor behaviors (grade and invasiveness) and clinical courses (recurrence, progression, metastasis and survival) with expression of FOXO3a were also be analyzed. Therefore, FOXO3a may be functioned as a useful prognostic marker for UCC patients' follow-up and medical care.

Disclosure of Interest: None declared.

P-253

A Case of Neck Lymph Node Metastasis from Urothelial Carcinoma with an Unusual Plasmacytoid Morphology in Fine Needle Aspiration (FNA)

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Objectives: To demonstrate a rare case of neck lymph node metastasis with the origin of urothelial carcinoma, focusing on the difficulty in diagnosis in fine needle aspiration (FNA) because of a distinct plasmacytoid morphology.

Materials and Methods: An enlarged neck lymph node was noted recently in a 62-year-old female patient with an underlying urothelial carcinoma, which was diagnosed half a year ago. Her urothelial carcinoma underwent a treatment of transurethral resection of bladder tumor (TURBT) and intravesical infusion of mitomycin. Fine needle aspiration of the neck lymph node revealed a hypercellular smear of monotonous cell clusters with mild discohesiveness and frequent single cell pattern. Those tumor cells manifested hyperchromatic and pleomorphic nuclei with prominent plasmacytoid features. Scant lymphoglandular bodies were present in the background. Since the morphology could not fit any common tumor over head and neck region, a metastatic poorly differentiated adenocarcinoma is suspected. Although neck lymph node metastasis of urothelial carcinoma was rare, the possibility should be excluded before searching for another primary tumor. Review of previous urine cytology and histology slide was performed by two pathologists and two cytotechnologists. Immunohistochemical (IHC) study was also applied.

Result: Comparing with the previous urine cytology, all of the reviewers agreed with the similarity of tumor cells in neck lymph node and urine specimen, though the morphology was unusual for urothelial carcinoma. We routinely had a departmental consensus record for all malignant cytology cases, in which two of us signed out the case as carcinoma and the others thought as high grade urothelial carcinoma. The histology slides of resection specimen showed an urothelial carcinoma with an uncommon inverted growth pattern and single plasmacytoid cell infiltration, extending to muscularis propria. A poor prognosis of urothelial carcinoma with such features had been reported. The IHC results were also compatible with each other. Both of them were positive for CK 20, CK7, Gata-3, and CD138.

Conclusion: We demonstrate a rare case of neck lymph node metastasis from an uncommon growth pattern and plasmacytoid cytological features of urothelial carcinoma, which leads to difficulty in diagnosis in cytology. The perfect match of morphology and IHC stains prove its urothelial origin. The clinical significance of plasmacytoid features and immunoreactivity of CD138 in tumor behavior needs to be determined.

Disclosure of Interest: None declared.

P-254

Cutaneous Metastases: A Study of 138 Cases Diagnosed by Fine Needle Aspiration Cytology

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Objective: Cutaneous metastases from internal malignancies occur in 0.8–5% cases and herald a poor prognosis. These may be the first sign of a clinically silent visceral cancer. The clinical presentation of the skin metastasis is often variable, non-specific and subtle. This is a clinico – cytologicalstudy of metastatic tumors in the skin and subcutaneous tissue.

Materials and Methods: A retrospective analysis was made of 138 patients diagnosed with cutaneous and subcutaneous metastasis on FNAC during the last 10 years. Primary tumors of the skin/subcutis were excluded from the study. Records of all patients were reviewed for their clinical history, local examination, a diagnosed primary tumor and time lag between skin lesion and primary tumor. The FNAC findings were correlated with the histologic findings in cases where the primary tumor was known.

Results: Of 138 patients, the primary tumor was known in 101 cases and in 37 cases the cutaneous deposit was the first manifestation of an unknown primary. Age of the patients ranged from 5 to 86 years and there were 76 (55.1%) males and 62 (44.9%) females. Clinically the most common lesion was a single nodule seen in 77 (55.7%) cases, multiple nodular lesions in 33 (23.9%), plaque lesions in 8 (5.7%) and big firm to cystic lesions in 20 (14.4%) cases. Chest wall was the predominant site involved in 53 (38.4%) cases, followed by abdominal wall in 33 (23.9%). In males, the most common primary site of tumor was lungs in 16 cases followed by gastrointestinal tract in 12. In females the most common cancer to metastasize to the skin was carcinoma breast in 23 cases followed by ovary in 6 cases. On cytological examination the most common diagnosis rendered were metastatic adenocarcinoma in 41 (29.7%), infiltrating ductal carcinoma in 23 (16.7%), and squamous cell carcinoma in 22 (15.9%) cases. Out of 37 cases with unknown primary, FNAC helped locate the primary site in 17 (45.9%) cases. In 20 cases the primary site of malignancy remained undiagnosed.

Conclusions: FNAC is a minimally painful, rapid and safe technique which can be used as a first line of investigation for confirmation of metastatic lesions to the skin. Critical evaluation of cytomorphological features along with relevant clinical details often allows an accurate diagnosis of an unknown primary in case of cutaneous metastases.

Disclosure of Interest: None declared.

P-255

8 Cases of Kaposi's Sarcoma Were Diagnosed by a Modified Fine Needle Aspiration Method Combing Cell Block in Chinese Acquired Immunodeficiency Syndrome Patients

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Objectives: Kaposi's sarcoma (KS) is a rare vasoformative mesenchymal tumor, which was mostly occurred in Acquired immunodeficiency syndrome patients and multifocal involving skin and other organs. The human herpesvirus 8 (HHV-8) is a known cause of KS. There was a little of publications in the diagnosis of Kaposi's sarcoma by fine needle aspiration cytology (FNA) and cell block method. This study is to evaluate the value of FNA combing cell block method in the diagnosis of Kaposi's sarcoma.

Patients and Methods: 471 cases of HIV positive patients in our FNA clinic were reviewed in this study. A modified method of FNA and cell-block preparation were employed. The key modification is using an auto-vacuumed syringe and it benefited the sampling more materials. Thus it also helped to make cell block easily. Immunohistochemistry stain, such as CD31, CD34, Eight factor, HHV-8 and SMA, was based on the cell block section.

Results: 8 cases (1.7%) of KS were diagnosed in this cohort. They were all male and the mean age was 38.5 years. 3 cases were from subcutaneous nodule, 2 were from oral mucosa, 3 were from lymph node (2 cervical and 1 inguinal). In the smears, KS showed hypercellular features consisted of plump spindle cells in a hemorrhagic background. It was mimic as low-grade spindle cell neoplasm. With cell block section, the vasoformative architecture was easily to recognize. The immunohistochemical study showed expression of CD31 (6 cases), CD34 (5 cases), Eight factor (5 cases) and HHV-8 (4 cases) in the atypical spindle cells.

Conclusions: FNA combing cell block is a valuable method in the diagnosis of Kaposi's sarcoma and it was an mini invasive procedure and especially amenable for AIDS patients. With this modified FNA and cell block procedure, the accurately diagnosis could be made with specific immunohistochemistry stains.

Disclosure of Interest: The Presenter does not have any commercial or associative interest that represents a conflict of interest in connection with the work.

P-256

Cytomorphologic Features Useful for Separation of Squamous Cell Carcinoma from Benign Squamous Cysts: A Multivariate Analysis

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Objectives: Fine-needle aspiration (FNA) is commonly used for the investigation of neck masses including branchial cleft cysts and cystic squamous cell carcinomas. The separation of these lesions has high clinical significance. While branchial cleft cysts occur predominately in children and young adults, carcinomas occur predominately in older adults. However, definitive separation based solely on patient age cannot be made. Cytologic separation of well-differentiated squamous carcinomas from benign cysts can be difficult. Few studies have statistically analyzed criteria useful for separation of these lesions.

Materials and Methods: We assessed inter-rater agreement for 19 morphologic features (nuclear, cytoplasmic and architectural) potentially useful for separation of squamous cell carcinoma from benign squamous cysts. The case set included 21 carcinomas and 13 branchial cleft cysts. Chance-corrected agreement was measured with kappa statistic. Associations between categorical variables were assessed using binary regression. Statistical calculations for agreement and strength of associations were calculated using Stata 14 (Stata LCC, College Station, TX).

Results and Conclusions: Actual agreement averaged 72% (range: 47–92%). Expected agreement averaged 64% (range: 32–97%). Chance-corrected agreement averaged 0.17 (range: –0.14–0.59). Acceptable chance-corrected agreement (i.e. >0.4) was observed for increased N/C (nuclear/cytoplasmic) ratio and pyknotic nuclei. The average prevalence of features ranged from 2% to 97%.

Recursive portioning suggested the following rule to discriminate benign from malignant cells:

Malignant: if increased N/C ratio (>2). Benign if small clusters are absent and no cells with increased N/C ratio present.

The positive and negative predictive values for the rule are 91% (95% CI: 76–98) and 87% (95% CI: 60–98). The area under the ROC curve for the rule is 87.5% (95% CI: 60–98%).

Separation of cystic well-differentiated squamous cell carcinoma from benign squamous cysts can be cytomorphologically dif-

ficult. Our multivariant analysis shows that best separation is achieved by analysis of N/C ratio and the presence or absence of small clusters of cells. Increased N/C ratio (>2) and the presence of small clusters of cells support a diagnosis of carcinoma with a predictive value of 91% (95% CI: 76–98). Good rater reproducibility was seen for N/C ratio (kappa = 0.47).

Disclosure of Interest: None declared.

P-257

A Case of Malignant Peritoneal Mesothelioma Diagnosed by EUS-FNA

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Malignant mesothelioma is an uncommon tumor arising from mesothelial cells lining the pleura, peritoneum, pericardium and tunica vaginalis testis. Malignant mesotheliomas showed frequently diffuse circumferential thickening, and less commonly nodular tumor extension. We report a case of malignant peritoneal mesothelioma diagnosed pre-operatively by endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA).

A 47-year-old man, who had worked at a car parts manufacturer for 8 months at the age of 18, presented left lower abdominal pain and tenderness on pressure. No mucosal abnormality was observed by gastrointestinal endoscopy. However, because of his continuous abdominal bloating, computed tomography (CT) scan was performed, which revealed multifocally nodular masses in his abdomen with a small amount of ascites. One of the nodules was attached to the rectum, for which we performed transrectal EUS-FNA. The obtained tissues were routinely processed for histology, and the remaining specimens in saline solution were processed for cytology. Cytological smears were stained with Papanicolaou stain, May-Grünwald-Giemsa stain and periodic acid-Schiff (PAS) stain.

Cytological smears were composed of single atypical cells and cohesive clusters arranged in three-dimensional structures and sheets. The cells showed oval to polygonal in shape with abundant cytoplasm and had occasionally cytoplasmic vascuoles. The nuclei were round to oval, and had fine granular chromatin, mild to moderate nuclear atypia and prominent nucleoi. There were occasionally bi- and multi-nucleated cells. The histological findings confirmed the presence of a malignant tumor whose cells were similar to those in the cytological smears and showed partially stromal invasion. Immunohistochemically, the tumor cells showed positivity for cytokeratin 5/6 (CK5/6), cytokeratin 7 (CK7), podoplanin (D2-40), HBME-1, calretinin, Wilms' tumor protein-1 (WT-1) and vimentin. They were negative for cytokeratin 20 (CK20), thyroid transcription factor-1 (TTF-1), napsin A, Ber-EP4, and CDX2. Thus, we considered a diagnosis of epithelioid malignant mesothelioma. Following the diagnosis, the patient underwent cytoreductive surgery and received post-operative chemotherapy.

It was a rare case of malignant peritoneal mesothelioma showing multiple nodular growth pattern. EUS-FNA is a safe and less invasive technique to obtain diagnostic materials, which is a useful approach to diagnose malignant peritoneal mesothelioma in such cases. To our knowledge, there are few reports of malignant peritoneal mesothelioma diagnosed by EUS-FNA on PubMed.

Disclosure of Interest: None declared.

P-258

Cytodiagnosis of Primary Lymphoma of the Bone: Report of 4 Cases with Histological Correlation

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Objectives: To study the clinical presentation and cytomorphology of primary lymphoma of bone, its histopathological correlation, incidence, site and pattern.

Materials and Methods: The study was conducted in the Department of Pathology, Goa Medical College over a period of 4 years from 2012 to 2015. 4 cases of primary bone lymphoma which comprised of 2 cases from spine, 1 from clavicle and 1 case of skull vault were studied. The age distribution of the cases ranged from 20 to 55 years, and comprised of 3 females and 1 male. Imaging modalities were used. The FNA smears were stained with hematoxylin – eosin and giemsa. The histological correlation was available in 3 out of the 4 cases. Sections were stained with hematoxylin-eosin.

Results: The FNA smears from all the 4 cases revealed hyperchromatic round cells, some showing prominent nucleoli, some with indented nuclei, background showed small mature lymphocytes and apoptotic bodies. The histopathological picture of the 3 cases, in which incision biopsy was done, revealed diffuse infiltration of fibrocollagenous tissue by a neoplastic lesion comprising of sheets of large atypical lymphocytes exhibiting significant nuclear atypia, high mitosis and apoptosis. Immunohistochemistry revealed tumor cells positive for CD 20, admixed lymphocytes positive for CD 3, MIB-1 >50%. Imaging from all the 4 cases revealed revealed permeative destruction of bone, with absence of any other mass at any other site than the site of presentation. Hence, Biopsy and immunohistochemistry confirmed the diagnosis of primary diffuse large B-cell lymphoma of the bone.

Conclusion: FNAC when done alone has a limitation in categorization of high grade lymphomas, however it can provide a rapid differential diagnosis, hence, leading to an early management of the patient. In our study all the cases were diagnosed as Primary diffuse large B cell lymphoma of the bone. Primary diffuse large B cell lymphoma should be considered as diagnosis and differential diagnosis of skeletal lesions.

Disclosure of Interest: None declared.

P-259

Oncocytic Neoplasm: Fine Needle Aspiration on Diagnosis

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Objective: To describe the findings in fine needle aspiration (FNA) of Oncocytic neoplasm of thyroid and parotid.

Materials and Methods: Three patients presented to outpatient department in Goa Medical College (three years study) having swellings of parotid, thyroid were examined and aspirated by FNA whose clinical diagnosis was thought to be a neoplasm. Out of the three, one had presented with both swelling of thyroid and parotid. Other had presented with swelling of thyroid and multiple cervical and mediastinal lymph nodes. And third patient had presented with a solitary thyroid nodule. All three underwent FNA followed by histopathological confirmation.

Result: Smears from the swelling in parotid and thyroid showed groups and sheets of Oncocytic cells containing abundant eosinophilic cytoplasm. Provisional diagnosis of Oncocytic neoplasm was given. Histopathology confirmed diagnosis of Oncocytic neoplasm of parotid with colloid adenomatous goiter, Oncocytic cell carcinoma of thyroid with metastasis to cervical and mediastinal lymph nodes and Oncocytoma of thyroid respectively in three patients.

Conclusion: FNA can yield diagnosis of Oncocytic neoplasm and allows early surgical therapy.

Disclosure of Interest: None declared.

P-260

Cytology in Diagnosis of Intraocular Malignancies: 20-Year Experience in a Single Institute

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Objectives: Diagnostic cytologic test is usually not required for ophthalmists to initiate a definite cancer treatment. However, in clinically ambiguous cases, cytology is useful in guiding the following clinical management. Nevertheless, intraocular cytology is usually a diagnostic challenge due to the small lesion size and the limited cellularity of the specimen. The aim of this study is to evaluate the utility of cytology in diagnosis of intraocular malignancies in our institute.

Materials and Methods: A total of 236 intraocular cytology tests in the period of 20 years from 1995 to 2015 were identified from the computer database at Taipei Veterans General Hospital. All the diagnostic categories were analyzed, including inadequate, negative, atypical, suspicious and positive. Follow-up clinical or pathological diagnosis was recorded from the medical chart and compared with the original cytology interpretation.

Results: The number of inadequate, negative, atypical, suspicious, and positive cytology tests was 26, 168, 8, 9 and 25 respectively. There were 28 patients has more than 1 (up to 5) cytology tests. Follow-up clinical or pathological diagnosis was available in 165 patients with a malignancy rate of 19.4% (32/165). Taken the most severe result into account in each patient with multiple tests, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 'positive' cytology tests in detecting intraocular malignancies was 59.3%, 100%, 100% and 91.1%. When we grouped 'atypical', 'suspicious' and 'positive' together as positive tests, the sensitivity, specificity, PPV and NPV became 75%, 96.2%, 82.8% and 94.1%. Among the intraocular malignancies, there were 14 lymphomas (10 central nervous system large B-cell lymphomas with intraocular involvement, 3 lymphomas of other sites with intraocular involvement and 1 primary intraocular lymphoma), 8 melanomas, 5 retinoblastomas and 5 metastatic carcinomas (5 of lung primary and 1 of unknown primary). The sensitivity of 'atypical and above' cytology test in detecting melanomas was the lowest (62.5%), following by retinoblastomas (80.0%), lymphomas (92.9%) and metastatic carcinoma (100%).

Conclusions: Cytology is a very specific test in detecting intraocular malignancy. The sensitivity could be improved if we group 'atypical and above' as positive tests without compromising the specificity. However, a false negative result is occasionally inevitable in some cancers, especially melanomas. Coupling with clinical findings, cytology remains a valuable tool in diagnosis of intraocular malignancies.

Disclosure of Interest: None declared.

P-261

The Use of Fine-Needle Aspiration Biopsy (FNAB) in the Diagnosis and Management of Patients with Uveal Melanoma

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Objectives: Uveal melanomas are rare tumors. Approximately 50% of patients develop metastasis and mortality rate is high even with current therapy. Treatment decisions are based on clinical examination and ocular imaging studies. FNAB can play important role in cases with atypical or undetermined clinical presentations. It also allows tumor sampling for prognostic testing (chromosome analysis and gene expression profiling), which helps to guide patient management. Herein, we review our cases of uveal melanomas diagnosed by FNAB.

Material and Methods: Computer based search of the pathology files, January 2002 to November 2015, identified cases diagnosed as uveal melanoma by FNAB in our department. The clinical findings, morphological features, results of prognostication tests and follow-up were reviewed.

Results: Specimens were from 11 male and 5 female patients, mean age 65.4 years (range 51–83 years). Ten specimens were from the right eye, and 6 from the left. Most specimens were obtained by trans-scleral FNAB using 20–25 gauge needles. Majority of patients (11) presented with blurred or diminished vision, while remaining 5 patients complained of floaters and/or light flashes. In 7 patients with unusual findings on exam, FNAB was performed to confirm diagnosis of uveal melanoma; 4 of these patients proceeded to have enucleation. On the remaining 9 patients, FNAB

was performed prior to brachytherapy to collect material for diagnosis and prognostication. Cellularity was adequate for diagnosis in all cases. In 9 cases a specific cell type was determined (2 spindle, 3 epithelioid and 4 mixed cell types); on 7 cases it was not specified. Fluorescence in situ hybridization (FISH) for chromosome 3 abnormality was performed in 7 cases: 3 cases had normal results and 2 had monosomy 3; in 1 case there were not enough cells for testing (minimum 200 cells), and on another case, the results were equivocal. Gene expression profiling was performed in 9 cases, and reported as class 1 (low-risk) in 7 cases and class 2 (high-risk) in 2 cases. No complications related to the FNAB procedure were described. Nine patients were alive and free of disease (mean follow-up, 49 months), 4 patients died of disease (mean follow-up, 21 months); 2 patients died without disease (6 and 112 months follow-up); one patient is alive with evidence of metastatic disease (37 months follow-up).

Conclusion: Our study supports that FNAB is a safe and effective technique to confirm diagnosis in patients with intraocular uveal melanoma, and it can be used to obtain tumor sampling for molecular genomic analysis. Such testing helps to determine patient's risk to develop metastases and prognosis. As novel target therapies for uveal melanoma become available, it will be critical to identify the high risk cases.

Disclosure of Interest: None declared.

P-262

Non-Hematopoetic Malignancies in Cerebrospinal Fluid: An Institutional Experience

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Objective: Demonstration of tumor cells in the cerebrospinal fluid (CSF) is essential for definitive diagnosis of leptomeningeal carcinomatosis. The aim of this study is to review non-hematopoetic malignancies detected in CSF.

Materials and Methods: We searched our database between 2006–2015 and identified 149 positive tests for non-hematopoietic malignancies. The cases positive or suspicious for malignancy were included. All cases had cytocentrifuge slides stained both by MGG and PAP stains When enough material was avaliable, unstained slides had been reserved for ancillary tests.

Results: 149 CSF spesimens from 73 patients were reviewed. Primary central nervous system (CNS) tumors accounted for 26% (n = 18) and among those, pediatric tumors such as medulloblastoma (n = 8) and retinoblastoma (n = 4) were comprising the majority. The others were as follows: ependimoma (n = 2), pinealoblastoma (n = 1), glioblastoma (n = 1), atypical teratoid/rhabdoid tumor (n = 1), and germ cell tumor (n = 1). For the metastatic tumors, the most common sites of the origin were the breast (28.8%), lung (13.7%) and stomach (6.8%). The others were as follows: 1 malignant melanoma, 1 colon carcinoma, 1 nasopharyngeal carcinoma, 1 ovarian serous carcinoma, 1 cervix squamous cell carcioma and 13 carcinoma not otherwise specified. Immunohistochemical studies were performed in 18 cases either for differential diagnosis between carcinomas and hematolymphoid malignancies or for identifying the primary site of the metastatic tumor. In 14 cases, immunohistochemistry helped achieving a conclusive diagnosis. CSF involvement was the first sign of malignancy in one case with positive TTF-1 staining in which lung mass was detected subsequently. In another case with two known primaries, immunohistochemistry made the metastatic primary apparent. In five cases, distinction between carcinoma and lymphoma couldn't be made because of the lack of clinical data and/or conclusive immunohistochemistry.

Conclusion: Metastatic tumors are seen more frequently than primary CNS tumors in CSFs. The patients frequently have a known primary which makes differential diagnosis easier. However, in selected cases ancillary tests are needed especially in patients either with more than one already known primary site or without history of cancer. Therefore, clinical history and obtaining sufficient material for ancillary tests is critical for the differential diagnosis.

Disclosure of Interest: None declared.

P-263

Osteoblastoma – A Rare Tumor with Unusual Presentation Diagnosed on Cytology

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Introduction: Osteoblastoma is a rare benign bone tumor comprising of <1% of primary bone tumors. It usually presents in the second decade of life with male preponderance. It is found commonly in the spine and long bones. It is rare to find osteoblastoma in the talus. Cytological diagnosis of osteoblastoma is very rare and only few cases have been reported in literature. The present case is being reported because of its rarity and unusual presentation in talus in elderly male which was initially diagnosed on fine needle aspiration cytology (FNAC).

Case Report: A fifty year old male presented with a progressively increasing swelling in the left ankle which was associated with pain for last two years. His X-ray showed destruction of the talus and lower end of fibula. FNAC was advised and the smears were cellular showing mononuclear round to spindle shaped cells with mostly having uniform nucleus and prominent nucleoli. Few osteoclastic giant cells were also present with transgressing capillaries and the diagnosis favoring osteoblastoma was made. The patient underwent excisional biopsy and the diagnosis of osteoblastoma was confirmed. The patient were subjected to surgery and now he is on follow-up.

Conclusion: The present case reports, a case of osteoblastoma, which is a rare tumor with unusual presentation in talus in an elderly male. The case also highlights the importance of FNAC in early diagnosis of osteoblastoma, which has been rarely used as diagnostic tool for osteoblastoma.

Disclosure of Interest: None declared.

P-264

The Investigation Report About Job Stress of the Cytologic Examination Workers in Japan

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Objective: The biggest cause of death for Japanese people is 'cancer' from 1981 to present day. The person who discovers and diagnosis of cancer is the cytologic examination professional. Cytotechnologists, pathology technicians, doctors and office workers all work together to handle cytologic examinations. The number of cytology board-certified medical doctors is 3370 and cytotechnologists is 8584 (2015 Dec.). They have been entrusted with an important mission in many areas with limited human resources. However, the number of reports about their mental health is limited. The purpose of this study is to carry out survey focusing on the mental health of cytotechnologists, pathology technicians, doctors and office workers engaged in cytologic examination and to discuss ways to improve it.

Materials and Methods: From January through April 2012, a total of 503 medical workers involved in cytologic examination were asked to answer a self-reporting questionnaire using BSCP/BSJS (in Japan) and to provide a free description.

Result: Cause of stress which felt most strongly is follows; cytotechnologists were reported as 'Quantitative workload'; pathology technicians reported 'Qualitative workload'; doctors reported 'Family and friends support'; and office workers reported 'Job control'. The following answers were obtained as characteristic of regard to strategies used to coping with stress; cytotechnologists: 'Refreshing change'; pathology technicians: 'Consult in order to solve the problem'; doctors: 'Changing of viewpoint'; office workers: all of the above. 'Presence of stress experienced' did not differ among the occupations, and all of job types reported this on the survey.

Conclusion: Even for similar work, we found that stress and the way of coping differed depending on the job type. These results are beneficial for the development of the medical field, and the need of continued research is suggested.

Disclosure of Interest: None declared.

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