risk factors do not lead to hyperaggregation of platelets. As also shown by previous data the role of platelets in venous vascular diseases may be underestimated and so far not totally understood. These investigations underline the significance of platelet hyperaggregation in patients with clinically apparent and spontaneous venous thrombosis. We therefore suggest to also include platelet function controls in screening programs for thrombophilia.

PS210

Recombinant platelet glycoproteins for the characterization of alloimmune thrombocytopenia

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The diagnostic characterization of alloimmune thrombocytopenic syndromes relies on the availability of reference material for genotyping and for antibody detection. Since the availability of certain donors having rare genotypes/phenotypes is limited the generation of immortalized B-lymphocytes and recombinant platelet alloantigens could be alternatives to the cumbersome search for suitable blood donors. We, therefore, established Epstein-Barr virus transformed B-lymphoblastoid cell lines from individuals with almost all human platelet alloantigens (HPAs). The suitability of the DNA for reliable genotyping was recently evaluated in a national workshop with more than 20 participants. Full length cDNAs encoding the known platelet alloantigens were generated by site-directed mutagenesis. Subsequently, stable mammalian cell lines expressing HPAs on GPIa, GPIIb and GPIIIa were established by transfection with the respective allele-specific constructs. A panel of alloantibodies was analysed by a modified MAIPA protocol using stable transfectants and reference platelets to prove the specificity of our transfectants. Allotype-specific reactions were observed with all transfectants tested so far. Stable transfectants were additionally evaluated by flow cytometry with alloantibodies against HPA-1a. Unspecific binding was occasionally observed, most probably due to the presence of heterophilic antibodies in our sera. Furthermore, the use of stable transfectants carrying specific mutations may allow to dissect different alloantibody types. To elucidate the structural prerequisites for HPA-1a epitope formation a large number of HPA-1a antibodies was analysed with a mutated GPIIIa isoform lacking the long-range disulfide bond and compared with wild type GPIIIa. In this study, we could demonstrate that stable transfectants are suitable reagents for the characterization of antibodies in alloimmune thrombocytopenia. Three cases of maternal immunization against rare alloantigens could be resolved by this strategy.

PS211

Identification of novel receptors on platelets

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Purpose: Cellular functions can be investigated at the transcriptome level by identification of mRNA using microarray hybridization analysis. The presence of mRNA molecules in platelets is well established and might give new insights into the biological functions of platelets. In the present study we analyzed the diversity of mRNA molecules in platelets by using microarray analysis and focused on the identification of novel receptors at the protein level. **Methods:** Purified platelet RNA characterized by using microarray hybridization analyses of 28,683 human genes. From the receptor category we selected candidates for the investigation of protein expression. Monoclonal antibodies specific for interleukin receptors IL-2R, IL-4R, IL-6R, IL-8R, IL-10R, IL-12R and IL-18R as well as chemokine receptors CCR1, CCR3 and CCR5 were used in flow cytometry. Some of the positive genes in the category of hypothetical proteins were further characterized by computer analysis of the protein sequence. **Results:** The microarray analysis revealed a total number of 5,310 (18.5 %) positive genes. In the category of receptors 221 of 1,056 (20.9 %) investigated genes were detectable. Interestingly, none of the 30 interleukin genes showed positive results but 10 of the 22 IL-receptors could be identified at the RNA level. The protein expression of IL-4R, IL-10R, IL12-R, IL-18R and CCR5 could be confirmed by flow cytometrical analysis of 12 individuals. The category of hypothetical proteins included 3,774 genes of which 654 (17.3 %) were positive. Further characterization of the predicted proteins of 72 genes by computer-assisted analysis revealed structural features of transmembrane receptors in 6 cases. **Conclusions:** Platelets contain a number of gene transcripts encoding receptors or receptor-like proteins. The interleukin, chemokine and cell adhesion receptors may be important for the role of platelets in inflammation. These membrane proteins may also carry novel alloantigens.

PS212

Lack of protein biosynthesis in leukocyte depleted platelet preparations

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PURPOSE: Platelets release a variety of preformed and stored factors upon activation, which are implicated in mechanisms of hemostatic, wound healing and inflammatory processes. Discussing sustained effects, the possibility of *de novo* protein biosynthesis in platelets may become relevant. In order to this, we performed experiments to demonstrate the role of protein biosynthesis in platelets. We focussed on IL-1□ and VEGF because of reports showing the biosynthesis of these factors and the presence of mRNA, respectively. To exclude leukocyte effects we also looked on leukocyte contamination. **METHODS:** Aliquots of platelet concentrates from pooled buffy coats were used unfiltered, one- and threefold leukocyte-filtered. The platelets were washed and activated with 1.0 U/mL thrombin. After removal of the releasate, $1.4x10^9$ activated platelets were incubated in cell culture medium and the amount of IL-1ß and VEGF was measured in the platelet pellet extract as well as in the medium supernatant by ELISA. Further protein labelling experiments with [35S]-methionine were performed and analyzed by 2D-gel electrophoresis and autoradiography. **RESULTS:** Nonfiltrated platelet samples expressed significantly increased levels of IL-1ß and VEGF 14 h in comparison to directly after activation. Delayed release effects were ruled out by testing the medium supernatant. Furthermore, puromycin inhibited the generation of IL-1□, indicating the involvement of translation activity. However, this increases of IL-1□ and VEGF concentrations were fully abolished by leukocyte depletion. The dependency of protein biosynthesis on leukocyte contamination was also observed when the incorporation of [35S]-methionine was tested. In contrast to unfiltrated platelets, no incorporation of [35S]-methionine into proteins was observed when leukocyte-depleted platelets were used. **CONCLUSION:** No significant protein biosynthesis activity in leukocyte-depleted platelets from healthy human adults were observed. Discrepancies to published results may reflect problems with leukocyte contamination. Probably, there is no relevance of protein biosynthesis for the physiological effects of platelets.

PS213

The nucleotide transporter MRP4 (ABCC4) is highly expressed in human platelets and located in granules, indicating a role in mediator storage

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Platelet aggregation is initiated by the release of mediators as ADP stored in platelet granules. Possible candidates for transport proteins mediating the accumulation of these mediators in granules include MRP4 (ABCC4), a transport pump for cyclic nucleotides and nucleotide analogs. We investigated the expression of MRP4 in human platelets by immunoblotting using two MRP4-specific antibodies which both detected a strong signal at 170 kDa. MRP4 function, assayed with [^{3}H]cGMP, could be detected in a low density membranous fraction containing plasma membrane proteins and in a fraction of higher density with intracellular granules. This transport was inhibited by dipyramidole and indomethacin with IC_{50} values of 12 and 22 µmol/L, respectively. Immunofluorescence microscopy revealed the abundance of MRP4 in intracellular granules which co-localized with the accumulation of mepacrine, known to be transported into delta-granules. An altered distribution of MRP4 was observed in platelets from a patient with

Hermansky-Pudlak syndrome with defective delta-granules. Transport studies with [^{3}H]ADP indicated in addition the presence of an orthovanadate-sensitive ADP transporting system, inhibited by dipyramidole and cyclic nucleotides. The results indicate a function of MRP4 in platelet mediator storage especially in the transport of ADP into dense granules. Inhibition of MRP4 may represent a target for anti-platelet therapy.

PS214

Subcutaneous administration of Anti-D in autoimmune thrombocytopenia

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Purpose: Since the first report in 1983, intravenous (i.v.) anti-D is increasingly used in the treatment of ITP although acute haemolytic reactions seem to take place in quite a few cases. In fact, we recently observed acute adverse reactions in two adult patients, and decided to investigate whether subcutaneous (s.c.) delivery could produce the beneficial effect of i.v. anti-D without these sequelae. **Patient and Methods:** Until now, a total of 12 patients (three childs and nine adults) have been studied so far. After obtaining informed consent, the patients, all Rh D-positive, received 50 µg/kg anti-D (Rhophylac®, ZLB Bioplasma, Bern, Switzerland or Rhesogam®, Aventis Behring, Marburg, Germany) within 3–5 minutes either intravenously or subcutaneously. Patients who received i.v. anti-D did not receive s.c. anti-D as long as their platelet count did not fall significantly below 30 x 10^3/µl, and s.c. injections were not administered at least four weeks following the last i.v. dose. **Results:** Whereas i.v. treatment was associated with signs of acute haemolytic reactions in three patients, the s.c. injections were well tolerated by all patients, and did not lead to significant haemolysis in a single case. In addition, s.c. treatment was repeatedly effective in all patients who required retreatment. **Conclusion:** Subcutaneous application of anti-D is as effective as intravenous application and it is associated with no or fewer complications than i.v. anti-D. Consequently, patients treated with s.c. anti-D must not be monitored as closely for signs and symptoms of acute haemolysis as those treated with i.v. anti-D. In addition, subcutaneous doses of >50 µg/kg can be tolerated if necessary.

PS215

The efficiency of maternal platelet concentrates versus highdose-donor-platelet-concentrates in fetal alloimmune thrombocytopenia (FAIT)

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Purpose: Fetal alloimmune thrombocytopenia occurs, when maternal human platelet antibodies (HPA),attach to fetal platelet surface possessing the responding antigen. In 10-20% of the affected cases intracranial haematoma has to be expected. Serial fetal blood sampling (cordocentesis) and aggressive platelet transfusion in short intervals appear to be an effective prenatal treatment to increase fetal platelet counts in thrombocytopenic fetuses reliable. **Patients, Materials and Methods:** Two groups, affected by FAIT were analysed. The first group were treated by maternal harvested and intrauterine transfused HPA-compatible platelet concentrates, on the other hand 46 fetuses were treated with selected-donor-platelets. The fetal thrombocytopenia was validated by umbilical blood sampling (cordocentesis) under continuous ultrasound guidance and generally combined with intrauterine platelet transfusion. **Results:** Both, platelet concentrates raised reliable the fetal post-transfusion platelet counts to >100,000/mm³ unlike to alternative non-invasive methods (e.g. ivIgG-treatment). But the high-dose-donor-platelet transfused fetuses showed additionally a significant increase in post-transfusion platelet counts in comparison to maternal platelet transfused fetuses. The amount of the increase showed on an average 18% higher post-transfusion platelet counts. No procedure related loss rate was observed. **Conclusion:** If serial platelet transfusions are to be administered, then every effort should be done to minimize the risk of procedure-related loss by referral to a centre with recognized experience in the management of F/NAIT. An important point is the possible exsanguation from the puncture site on the cord as described in some publications. Our study confirms that the risk of the fetal bleeding from transfusion procedure is lower than the risk of intracranial haemorrhage from a low fetal platelet count, i.e. if the fetal thrombocytopenia does not respond maternal ivIgG-therapy. Intrauterine platelet transfusions in fetal AIT may be associated with fetal birth distress, which can be avoid by delivery at around 36 weeks of gestation.

PS3: Pathogen-Inactivation and Safety Aspects of Hemotherapy

PS301

Cost-benefit ratio and costs per prevented release of an infectious blood product for blood donation screening tests

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Purpose: As resources of the German public healthcare system decrease, the cost-utility ratio of therapies and measures has become a focus of discussion and cost per QUALY gained started to be a decision criterion for public health authorities. **Materials/Methods:** We calculated the cost of blood donation screening tests performed in our institute in 2002, taking into account costs due to reactive test results. For NAT Testing, we collected the data of all German Red Cross blood donation services concerning NAT-only positive donations. We also calculated the QUALYs lost due to the release of a blood product infective for HIV, HCV and HBV, taking into account efficiency of infection and average life expectancy of transfusion recipients. **Results:** Costs per prevented infection through ALAT screening were €306,698 (HAV infection), €99,486 for serologic syphilis testing, €20,138 for HCV-antibody test, €207,645 for HIV-antibody test and €8,384 for HBs antigen test. For NAT-Testing, costs per prevented infection without license fees were €3,418,765 for HCV NAT based on €3 cost per donation screened, €2,782,022 for HIV-NAT based on €1 per donation screened and €298,459 for HBV-NAT, based on €1 per donation screened. Costs per QUALY gained were €52,398 for HBs antigen test, €21,687€ for HCV-antibody test and €29,246 for HIV-antibody test when calculating a loss of QUALY of 0.6 for HCV infection, 7.1 for HIV infection and 0.16 HBV infection. For NAT-Tests, costs per QUALY without license fees were €5,697,942 for HCV-NAT, €391,834 for HIV-NAT and €1,865,371 for HBV NAT. When taking into account average life expectancy of recipients of blood products, costs per QUALY gained are even higher. **Conclusion:** Using our model, costs per prevented infection and costs per QUALY gained through blood donation screening test are very high. When taking into account the benefit of our screening tests in QULYs gained, tests like ALAT or serologic syphilis test, which usually are considered to be “cheap” turn out to be expensive.

PS302

Results of malaria screening for first time allogeneic blood donors

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The increase in tourism and migration causes an increase in returned travelers and immigrants from malaria endemic areas who are willing to donate blood. Therefore we need a cost-effective strategy for screening blood donors regarding the legislation of the European community. Between 01.07.2002 and 31.03.2004 we performed an IIFT (indirect immunofluorescence test) malaria screening for first time allogeneic blood donors willing to donate blood as a part of our predonation examination. 130 of these first time allogeneic donors out of 8403 had a history of travel in a malaria endemic

area with signs of fever of unknown origin in the last 12 months or a history of immigration 3 years before the predonation examination. 60/130 of these donors screened for malaria were not accepted: 13/130 donors had positive infectious markers for hepatitis and a positive malaria screening, 10/130 donors had positive infectious markers for hepatitis and a negative malaria screening, and 37/130 donors had negative infectious markers for hepatitis but a positive malaria screening. Regarding the donation frequency of the accepted malaria screened and not screened first time allogeneic donors there is no difference between these two groups. Our results render a high occurence of positive infectious markers for hepatitis in the group of first time allogeneic donors screened for malaria indicating a geographical congruence of hepatitis endemic areas and malaria risk regions. We conclude that a screening test such as the IIFT, which costs 11,90 Euro, can be done for donors with travel or immigration history. With regard to the shortage of blood reserves the IIFT screening can help to minimize unnecessary deferral of blood donors.

PS303

Hepatitis B Vaccination Status In Two Different Populations Of Blood Donors In Germany – Is It Time To Vaccinate?

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Purpose: As the risk of transfusion-transmitted hepatitis B virus infection (TT-HBV) still exists the introduction of HBV NAT testing is a matter of discussion today. Anyhow, another possibility of protection against TT-HBV is possible: active vaccination of blood donors (BD). No data are available displaying the rate of HBV vaccinated BD. We compared the HBV vaccination status and demographics of two BD populations, apheresis donors (AD) of a university based and whole blood donors (WBD) of a Red Cross blood donation service. **Methods:** Randomly incoming AD and WBD were requested to answer a standardized questionnaire. Therein it was asked whether they had been vaccinated against HBV and for what reason. Demographics of BD were registered. **Results:** The questionnaires of 260 AD and 6812 WBD were analysed. Whereas the distribution of gender was similar (p=0.372), AD were younger, had more academic social background, donated blood more often, and had higher HBV vaccination rate (table 1). HBV vaccination rate was lower for men and declined with increasing age. In both groups HBV vaccination was mainly related to work although for WBD foreign travel was nearly of similar significance. **Conclusions:** AD are significantly more often HBV vaccinated than WBD which seems to mainly due to different social background and age. As AD donate blood quite often a high proportion of HBV vaccinated AD might reduce the risk of TT-HBV. Therefore, one should rise the question whether HBV vaccination of non-immunised AD could be as effective as introducing HBV NAT testing.

TABLE 1. Demographics of the BD

		AD		WBD		P-value
Age (years; mean±SD)		32.9±10.3	N=260	40.7±12.6	N=6790	<0.001
Gender	Female	43.8%	N=114	46.6%	N=3174	0.372*
	Male	56.2%	N=146	53.4%	N=3637	
Number of blood donations (mean±SD)		38.5±43.1	N=253	17.5±17.7	N=6541	<0.001
Period of blood donation (years; mean±SD)		6.3±7.0	N=253	11.9±11.1	N=6159	<0.001
Number of blood donations per year (mean±SD)		7.9±6.3	N=253	1.6±0.9	N=5528	<0.001
HBV vaccinated		41.2%	N=260	21.7%	N=6807	<0.001

* not significant

PS304

Amotosalen / UVA – Light Treatment of Platelet Concentrates: Inactivation Capacity Towards Defined Strains of Three Bacterial Species and One Fungus

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Purpose: We evaluated the inactivation capacity of amotosalen / UVA-light treatment (PCT) towards selected bacteria and one fungus in pooled platelet concentrates (PCs). **Methods:** Prior to the study a great number of strains have been characterised for their ability to grow in PCs obtained from at least 50 different donors. For our investigations we have chosen one defined strain from the following: *Klebsiella pneumoniae* (as Gram-negative rod), *Staphylococcus epidermidis* (as Gram-positive coccus), *Bacillus cereus* (as spore former, isolated spores have been applied), and *Candida albicans* (as fungus) representing typical groups of microorganisms. In total 100 PCs derived from pooled buffy-coat platelets were contaminated either by ~ 10 Colony Forming Units (cfu) per bag followed by a storage period of 18 – 24 hours before PCT or by ~ 3.5 x 10^6 cfu per bag of the respective microorganisms followed immediately by PCT. Inactivation was evaluated immediately after PCT as well as during a storage time of 7 days by microbiological methods. The amotosalen / UVA-light treatment has been performed with the Intercept Blood System (Baxter Healthcare Corporation and Cerus Corporation, Deerfield, IL, USA) following the instructions provided by the manufacturer. **Results:** Different inactivation rates have been observed. 3 – 4 x 10^6 cfu of the applied strains of *Klebsiella pneumoniae* as well as of *Staphylococcus epidermidis* could be extinct by the Intercept Blood System treatment. In case of the *Candida albicans* strain, a reduction from 3 – 4 x 10^6 cfu to 1 – 2 x 10^4 cfu could be achieved, representing an inactivation rate of ~ 2.5 log steps. Spores of *Bacillus cereus* survived the procedure. **Conclusions:** Several studies about inactivation capacity of amotosalen / UVA-light treatment towards bacteria have been published. In our examination, especially characterised strains have been used. Our data concerning the strains of *K. pneumoniae* and *St. epidermidis* are generally comparable with the results published. *Candida albicans* represents the first fungus examined with the Intercept Blood System. Purified spores of *Bacillus cereus* (e.g. free of vegetative cells) have been studied for the first time.

PS305

Methylene Blue-Treated Plasma: Toxicological Profile of Methylene Blue and Its Photoproducts

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Purpose: The MacoPharma Theraflex® System uses methylene blue (MB) and visible light for virus inactivation of plasma for transfusion (MB-plasma). MB is added at a concentration of 1µM. After illumination most of the photosensitizer and its photoproducts are removed by an integrated depletion filter. A considerable number of toxicological data on MB are available in the literature. However, long-term studies are lacking. They are necessary because in certain indications MB-Plasma is administered for several weeks, for example in the treatment of thrombotic thrombocytopenic (TTP) patients. Recent studies with MB were conducted by the American National Toxicological Program (NTP). They focused on the endpoints: Short-term toxicity (4 and 13 weeks), conventional teratology and long-term carcinogenicity (2 years). However, in these studies MB was administered orally and therefore this application route differs from the mode of application used for MB-plasma (intravenous route). It was the aim of the present investigation to elucidate whether toxicological data from the NTP studies can be used to assess the toxicological properties of MB after intravenous administration. **Methods:** The adsorption, distribution and excretion of ^{14}C-labeled MB following oral and 24-h infusion, respectively, were investigated in rats. A nominal dose level of 20 mg/kg body weight was administered by

gavage or by 24-h infusion. The observation duration was 96 hours. The study was conducted according to GLP. **Results:** After oral as well after intravenous application a biphasic elimination of MB-derived radioactivity was detected. In both cases the half-lives of MB were very similar. After oral application t ½ α: 2.8 min (males and females),T½ β: 13.7 min (males), 18.4 min (females) and after intravenous application T½ α: 7.5 min (males), 2.8 min(females) and T½ β: 16.7 min (males), 11.4 min (females), respectively. The systemic bioavailability was almost complete with an average value of approx. 93%, indicating that the oral dose was well absorbed. Within 96 h the excretion of MB was almost complete in both groups. There was no tissue storage subsequent to either oral or 24-h intravenous administration. **Conclusion:** The data obtained indicate that the results of the NTP study can be used to assess the toxicological profile of MB following intravenous application.

PS306

Significance of Confirmatory Testing of Blood Donors with Positive Screening Findings for Anti-HIV and Anti-HCV

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BACKGROUND: By law, blood donors are screened for anti-HIV and anti-HCV antibodies on each blood donation. Positive screening findings need to be confirmed by alternative (confirmatory) tests to prevent inaccurate diagnosis and to assure correct donor counselling. **METHODS:** Retrospectively, we investigated over time blood donors with positive screening findings of anti-HIV/HCV and not-negative confirmatory testing (excluding donors with positive confirmatory testing) in terms of prevalence, sex distribution, surrogate marker findings and seroconversion. Screening/confirmatory tests for HIV and HCV were performed by using Murex HIV-1.2.0 Abbott / HIV Blot 2.2 genelabs from Abbott and HCV 3.0 ELISA Ortho / HCV RIBA 3.0 Immunnoblot from Ortho, resp. **RESULTS:** Over a 6 9/12 years period (January 1997 to October 2003) for HIV and over a 7 9/12 years period (January 1996 to October 2003) for HCV, 118 donors with not-negative HIV confirmatory test result (nnHIV) and 60 donors with not-negative HCV confirmatory test result (nnHCV) were observed. 65 (55%) of nnHIV donors and 27 (45%) of nnHCV donors followed the invitation for follow-up testing and constituted the study population. The main findings are summarized in the table.

Table: Blood donors' characteristics with not-negative findings for HIV and HCV

	HIV	HCV	p
No of not-negative (nn) donors (N)	65	27	
Prevalence per 100'000 donations	26	13	0.01
Sex predomination (%male)	69%	74%	n.s
Time interval $1^{st}/2^{nd}$ confirmatory test (CT) (mean days, range)	201 (18–2877)	274 (10–2341)	n.s
2^{nd} screening test (2^{nd} ST) negative	54%	41%	n.s
2^{nd} ST positive	46%	59%	n.s
2^{nd} CT negative	32%	22%	n.s
2^{nd} CT not-negative	68%	78%	n.s
2^{nd} CT positive	0%	0%	

CONCLUSIONS: 1. Only 50% of blood donors with ambiguous test findings are willing for additional testing at the same institution. 2. Over time, 22% - 32% of donors clear their ambiguous test findings and can be reinstituted as blood donors. 3. Positive conversion of donors with ambiguous test findings in either parameter have not been observed. 4. Ambiguity on blood donor screening for HIV/HCV constitutes a challenge for donor counselling but does not justify to stigmatize blood donors. 5. An extended test interval of 12 to 24 months between 1^{st} and 2^{nd} testing is recommended.

PS307

Feasibility of NAT screening of blood donors for Orthopoxvirus can potentially prevent dispersion of viral agents in case of bioterrorism

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BACKGROUND: Biological weapons are very attractive for terrorism. As a result of international collaboration under the WHO eradication program, smallpox was declared eradicated in 1980. Therefore the immunization programs were discontinued worldwide. Because most people are immunologically native, variola virus is considered to be a potential threat agent or bioterrorist weapon. Real-time PCR for orhtopoxviruses was developed for fast and safe analysis. Melting analysis following PCR enables the differentiation beween variola and other genotypes like vaccinia or camelpox virus. **STUDY DESIGN AND METHODS:** RealArt™ Orhtopox LC PCR Kit was used to amplify Orthopox virus (OPV) sequences from blood donor samples. We tested 31,500 blood donor samples in mini pools of up to 96 samples. To validate the sensitivity of the assay, routine donor mini pools (90 ± 6 samples per pool) were spiked with vaccinia virus and used as positive controls. **RESULTS:** Specificity was 100% because none of 31,500 blood donors were positive for OPV. The detection limit of the assay was 1.41 input copies/PCR. Therefore we calculated a sensitivity of 211.5 copies/mL. Overall, 0.28% of test results had to be considered invalid due to negative internal controls. **CONCLUSION**: The real-time Orthopox PCR kit enables detection of OPV in viremic blood donor samples even in the beginning of the disease when patients present minor clinical symptoms and could be implemented in routine screening procedure immediately. Thus the assay could potentially help to prevent dispersion of viral agents by blood transfusion in case of bioterrorism.

PS308

Transfusion-transmitted HBV infection by a chronic anti-HBc-positive low-level carrier

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Background: A recipient-directed look-back procedure was caused by a HBV-infection of a haematological patient. One of 22 transfused erythrocyte concentrates revealed anti-HBc positive. A donor-directed look-back procedure was started. **Methods:** All look back samples of the last 3 years were tested for HBsAg, anti-HBs, anti-HBc and HBV-DNA by single sample PCR (95% cut off at 8.6 IU/ml). The initially screening of donations was done with ABBOTT PRISM for HBsAg and with Minipool-NAT (95% cut off at 106 IU/ml in the single donation). **Results:** The clinically inconspicious male donor gave 15 whole blood-units since 2000. These donations were routinely screened negative for HBsAg and HBV-DNA. 13 of these 15 donations were transfused. All investigated look-back samples were negative for HBsAg but positive for anti-HBc. A seroconversion to anti-HBs was detected in one sample with a titre of 17IU/l. HBV-DNA was detected in 10 of 12 look back samples with viral loads ranging from 2,3 to 11,6 IU/ml. ALT was normal. HBV transmissions were caused by five erythrocyte concentrates as shown in table. All recipients of these five erythrocyte concentrates became anti-HBs- and anti HBc-positive.

Date of donation	Anti-HBc	Anti-HBs	HBV-Single Sample-NAT	HBV-Transmisson
30.08.03	Pos	Pos 17IU/l	2,3IU/ml	No *
14.06.03	Pos	Neg	5,3IU/ml	Yes
22.03.03	Pos	n.t.	Neg	No
11.01.03	Pos	Neg	4,7IU/ml	Yes
26.10.02	Pos	Neg	Neg	No
25.05.02	Pos	Neg	11,6IU/ml	Yes
02.03.02	Pos	Neg	9,1IU/ml	No *
15.12.01	Pos	Neg	2,4IU/ml	No
02.08.01	Pos	Neg	6,7IU/ml	No
05.05.01	Pos	Neg	3,7IU/ml	Yes
17.02.01	Pos	Neg	10,6IU/ml	No
25.11.00	Pos	Neg	7,7IU/ml	Not transfused
07.08.00	n.t.	n.t.	n.t.	No *
01.04.00	n.t.	n.t.	n.t.	Not transfused
15.01.00	n.t.	n.t.	n.t.	Yes

n.t. – not tested, * recipient deceased

Conclusions: The low viral titres in the HBV DNA-positive samples from the infectious anti-HBc-positive donor can cause HBV infektions. Such low titres can not be detected by minipool NAT. As HBsAg testing was never positive anti-HBc is the only marker to prevent transfusion-transmitted HBV infections caused by chronic HBV carriers.

PS309

The in-vitro Quality of ComTec™ Apheresis Platelet Concentrates using the Intercept™ Preparation Set and Blood System over an extended Storage Period of 7 days

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Purpose: Apheresis platelets have to be suspended in 53-68% additive solution (InterSol™, Baxter) when the pathogen reduction treatment with the Intercept™ Blood System is applied. When cell separators without automatic addition of InterSol™ are used, the plasma volume of the platelet concentrates has to be reduced by centrifugation (Intercept™ Preparation Set). Subsequently, platelets are resuspended in InterSol™ before the photochemical treatment is performed**. Methods:** First, this study evaluated the performance of the Intercept™ Preparation Set and Blood System with 12 apheresis platelet concentrates produced with the ComTec™ (Fresenius, Germany). Second, platelets' in vitro characteristic (pH, glucose, lactate, LDH, hypotonic shock response, aggregation with collagen) were followed over an extended storage period of 7 days.
Results:

	Day 2 before Intercept preparation	Day 2 after Intercept preparation	Day 2 after Intercept Blood System	Day 5	Day 7
Volume (mL)	375.9 (22.5)	300.2 (4.9)*	258.2 (6.5)*	231.8 (7.0)*	202.5 (7.0)*
Platelet dose (10^{11}/unit)	4.03 (0.38)	3.45 (0.33)*	2.88 (0.25)*	2.54 (0.26)*	2.20 (0.28)*
pH (22°C)	7.35 (0.10)	7.28 (0.04)	7.12 (0.04)*	7.07 (0.07)*	6.91 (0.11)*
Glucose (mg/dL)	362.3 (45.1)	146.5 (18.7)*	137.3 (19.4) *	80.3 (20.3)*	37.3 (17.9)*
Lactate (mmol/L)	2.4 (0.5)	1.9 (0.4)	2.2 (0.3)*	7.6 (0.5)*	11.8 (1.1)*
LDH (U/L)	134.3 (36.8)	74.0 (18.4)*	75.1 (15.1)	103.6 (19.5)*	111.7 (21.6)*
CD62p(%)	3.6 (1.4)	7.9 (3.5)*	10.2 (4.7)	19.3 (3.3)*	20.5 (7.9)*
HSR (%)	97.1 (6.1)	46.9 (7.9)*	43.5 (8.4)	59.0 (13.0)*	48.9 (16.4)*
Aggregation (%)	77.7 (7.0)	64.3 (5.2)*	38.5 (16.9)*	41.2 (18.1)*	20.6 (14.8)*

Results are given in mean and (standard deviation), *p<0.02 versus previous evaluation step (Wilcoxon Matched Pairs for day 2, and Friedman Anova for the days 2-7).

Conclusions: The Intercept™ Preparation Set used in combination with ComTec™ apheresis platelets is capable of producing platelet units meeting the requirements for Intercept™ Blood System. The whole processing steps of Intercept™ Preparation Set and Blood System cause a mean platelet dose loss of 16.5% which has to be considered for reaching satisfying platelet increments after transfusion. A gradual decline of platelets' in vitro characteristics is observed during storage of 7 days. The pH levels decrease after the treatment and during storage but well comply with international quality guidelines.

PS310

Results of validation procedures for photochemical treated platelet concentrates

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Purpose: The INTERCEPT Platelet System™ using psoralen S-59 and UVA illumination inactivates a broad spectrum of viruses, bacteria, and WBC in platelet concentrates (PC). We investigated the quality and functional integrity of platelets in buffy-coat PC (B-PC) and apheresis PC (A-PC) after INTERCEPT treatment and during storage. The study was part of a validation procedure in order to fulfil the requirements of the national authorities. **Methods:** Platelets were suspended in 35% plasma and 65% InterSol. After pathogen inactivation (150µM S-59 and UVA illumination (~3J/cm^2)), any excess of S-59 and unbound photoproducts were removed by a compound adsorption device. Platelets,WBC or RBC were quantified by hematology analyser and flow cytometry. Platelet biochemistry (pH, pO_2, pCO_2, Glucose, Lactat) and CD62P expression were assessed during storage of 7 days. Results: The results obtained for the quality assessment after INTERCEPT treatment are given below:

	PLT $\times10^{11}$/U	WBC $\times10^{6}$/U	RBC $\times10^{9}$/U	pH	
day	1	1	1	1	6
B-PC	3.1±0.3	<0.1	0.9±0.3	7.2±0.0	7.0±0.0
A-PC	3.1±0.2	0.2±0.3	0.3±0.2	6.8±0.1	6.6±0.1

	pO_2 (mmHg)		pCO_2 (mmHg)		Glucose (mg/dl)		Lactat (mmol/L)	
day	1	6	1	6	1	6	1	6
B-PC	111±20	127±14	34±2	24±2	150±4	44±9	3±0	12±2
A-PC	132±20	144±9	33±3	16±3	108±8	12±1	3±1	12±1

	CD62P (%)		CD62P PMA Stimulation (%)	
day	1	6	1	6
B-PC	33±7	47±3	94±1	81±6
A-PC	33±9	52±9	87±6	79±5

Conclusions: The results obtained for INTERCEPT treated PC meet the national and european requirements of quality assurance to ensure efficiacy and safety for platelet transfusions. There are significant differences (pH, Glucose) in comparison of B-PC and A-PC which might be explained by the smaller suspension volume of A-PC. The pathogen inactivation has no obvious influence on the in-vitro function of platelets. Furthermore, the INTERCEPT Platelet System allows for easy and safe pathogen inactivation in routine blood component manufacturing with a high grade of GMP conformity.

PS311

HCV or HBV NAT positive blood donors – what comes after Votum 24

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Purpose: In our institute, per year approximately 100 blood donors get the diagnosis of being infected with HCV or HBV. We wanted to monitor the course of these patients after diagnosis to find out if further diagnostic an therapeutic measures can be improved. **Methods / Results:** We investigated the outcome of blood donors who have been diagnosed being HCV or HBV PCR positive in 2002 by a questionnaire. Analysis of responding donors showed that most of the HBV/HCV positive donors describe their health status as being good or very good. Most donors knew that hepatitis B or C can be transmitted though blood transfusion (24/30), sex (24/30) or i.v. drug abuse (16/30). Although blood transfusion is no longer a frequent way of infection, it is still the most known way of HCV/HBV transmission. One of our donors thought, HBV/ HCV could be transmitted by handshake, and 3 out of 30 donors thought using public toilets could transmit Hepatitis B or C. Most of the HCV/HBV positive blood donors (24 out of 30) were not in-

formed on the existence of self-help groups. Five HCV or HBV positive donors told their physician about their Hepatitis B or C (figure 9). On the other hand, this means that 5 out of 30 donors did not inform their physician about their infection. This is probably the main reason why only few donors were admitted for sonography (12 out of 30), determination of viral load (18 out of 30) and determination of ALT (22 out of 30) after diagnosis. Only five out of 30 donors felt well informed about their disease, and 25 wanted to get the information brochure from the Kompetenznetz Hepatitis. Our data show that the incidence of HBV and HCV in blood donors is very low. Most of the positive donor samples in 2002 came from first time donations (HBV: 65 %, HCV: 49%),have been taken from donors willing to donate but without donation (HBV: 18 %, HCV: 18 %) or from donors for autologous transfusion (HBV: 5% HCV: 18%) Only 12% of the HBV NAT positive donors were repeated donors, and only 15% of HCV NAT positive donors were repeated donors. **Conclusion:** The lack of knowledge about he way, Hepatitis B or C can be transmitted and about possible specific therapy is very unsatisfying from an epidemiological point of view. Donor information and co-operation with physicians has to be intensified.

PS312

Two novel real-time RT-PCR assays for rapid detection of 23S rRNA and *gro*EL mRNA as an indicator of viable bacteria in platelet concentrates

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Bacterial contamination of blood components especially of platelet concentrates (PCs) with an incidence of approximately one per 2000 units represents a high infectious risk in blood transfusion. Conventionally the sterility testing of PCs with an automated bacterial blood culturing system is an time consuming process. Here we present two novel real-time RT-PCR assays carried out on the LightCycler instrument which are compared regarding specificity and sensitivity using different templates to detect a majority of the clinical important bacterial species in platelets. Primers and probes specific for the conserved regions of the eubacterial 23S rRNA gene or the *gro*EL gene (encoding the 60-kDa heat shock protein HSP60) were designed. In addition we discuss the problem of bacterial contamination of reagents that caused false positive-results during the development of the 23S rRNA RT-PCR. Treatment with 8-methoxypsoralen (8-MOP) and UV irradiation reduced the level of contaminating DNA. The sensitivity of the assays was influenced by the enzyme system which was used. Using r*Tth* DNA polymerase 500 CFU per mL of *E. coli* and *Staphylococcus epidermidis* were detected whereas the two enzyme system consisting of MMLV-reverse transcriptase and *Taq* DNA polymerase enables a lower detection limit of 16 CFU per mL. Using *gro*EL mRNA as the target of RT-PCR under optimized conditions 125 CFU per mL of *E. coli* were detected and no problems with false positive results caused by reagent contaminations or cross reaction with human nucleic acids could be found. Furthermore, the use of mRNA as an indicator of viability was demonstrated because of its short half-life in the bacterial cell.

PS313

Introduction of a new PCR based Mycoplasm Detection system

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PURPOSE: Mycoplasms pose a recurrent threat for cells grown in culture. Despite mycoplasma contamination of cell cultures effect the cellular metabolism dramatically there is little influence on cellular morphology and no tarnish of the media. Legal regulations according to the *European Pharmacopoeia* and ICH Guidelines for biotechnological/biological products demand frequent testing for the absence of Mycoplasma species. We developed a PCR-based method to detect Mycoplasms and designed a validation procedure to evaluate the PCR assay. **METHODS**: The PCR assay specifically amplifies a 280 bp target of the gene coding for the small ribosomal subunit. Bacterial DNA was isolated using a modified protocol for the Qiagen QIAamp DNA Blood Mini Kit. Amplification products were analyzed by agarose gel electrophoresis and documented by using a UV documentation system. After PCR optimization with Cosmid derived PCR templates we developed a validation concept according to the guidelines of the *European Pharmacopoeia*, section 2.6.7. The validation strategy consisted of the following steps: (1) cultivation of *Mycoplasma orale* and *Mycoplasma pneumoniae* in modified Hayflick medium, (2) determination of the colour changing units (CCU) in repeated dilution experiments as standard tests, (3) DNA extraction, (4) comparison of the PCR results to CCU values. The validation was performed with three independent runs. **RESULTS**: The PCR assay includes two amplicons: the test amplicon that is generated in the presence of Mycoplasma DNA and another smaller amplicon that serves as an internal control. The detection realm of the PCR system includes 26 mycoplasma species including the required test strains of the *European Pharmacopoeia*. Tests with samples contaminated with human DNA showed no cross-reactions or loss of sensitivity. The analytical sensitivity was determined by amplification of serial dilutions of bacterial culture, and the lower limit of detection for PCR was the CCU equivalent of 1000 for *M. orale* and *M. pneumoniae.* **CONCLUSIONS**: We successfully introduced and validated a PCR-based assay for the detection of mycoplasma species. This PCR method can be used as a new tool for release testing of cell culture generated therapeutics.

PS314

Distribution of ^{14}C-thionine and its photoproducts before and after Photodynamic and UVB treatment of plasma-reduced platelet concentrates

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Purpose: The photosensitizer thionine (Th) may be used for photodynamic inactivation of pathogens in platelet concentrates (PC). Th is fragmented during illumination. This property is identical to that of the psoralen compound amotosalen, which is used for the same purpose. That part of amotosalen and its photoproducts which are not bound to plasma proteins and platelets are subsequently removed by a special device (Transfusion (2004) 44(3):313-319). The same approach could be worthwhile for PC treated with Th/light and UVB. In order to assess this, the distribution of Th (before and after treatment) and its photoproducts between aqueous phase, platelets and plasmaproteins was investigated. **Materials & Methods:** Plasma-reduced PC in storage medium PAS-IIIM containing approx. 30% plasma were prepared from pools of 4 buffy coats. The pathogen inactivation procedure uses yellow light (94 J/cm^2) in the presence of 1µM Th, followed by irradiation with UVB (1.2 J/cm^2). Th used was either unlabelled or radiolabelled by ^{14}C. Determination of unlabelled Th was by HPLC. The distribution of ^{14}C-Th derived radioactivity was investigated by fractionating the PC by centrifugation and by precipitating the plasma proteins with trichloroacetic acid. Radioactivity was measured by liquid scintillation counting. **Results:** The distribution of radioactivity (% of the added ^{14}C-Th) between the different fractions of the PC (aqueous phase, platelets and plasma proteins) is shown in the following table (mean values, +/- SD, n=5):

Untreated PC	Treated PC
Free: 67.0+/-7.9	Free: 27.7+/-3.5
Bound to plasma proteins: 38.9+/-8.4	Bound to plasma proteins: 67.7+/-7.0
Bound to platelets: 0.3+/-0.0	Bound to platelets: 10.9+/-2.5

HPLC analysis revealed that there was almost no free Th after treatment. This suggests that most radioactivity found free (i.e. in the aqueous phase) after treatment consisted of photoproducts. **Conclusion:** Most Th and its photoproducts are bound to platelets or to plasma proteins after treatment. That part which remains unbound, i.e. in the aqueous phase (approx. 27%) can subsequently be removed by a suitable device. Supported by the Forschungsgemeinschaft der Blutspendedienste des Deutschen Roten Kreuzes

PS315

Patients with Congenital Coagulation Factor Deficiencies Demonstrate Consistent Therapeutic Responses to Repeated Transfusions of Plasma Prepared with Pathogen Inactivation Treatment (INTERCEPT Plasma)

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Purpose: Photochemical treatment using amotosalen HCl (S-59) and UVA was developed to inactivate pathogens and leukocytes in plasma (INTERCEPT Plasma). The present study evaluated the pharmacokinetics and hemostatic efficacy of INTERCEPT plasma. **Methods:** An open-label, multi-center trial was conducted in patients with congenital coagulation deficiencies (Factors I, II, V, VII, X, XI, XIII, and Protein C) to measure the kinetics of specific coagulation factors, hemostatic efficacy, and safety of INTERCEPT Plasma. Prothrombin time (PT), partial thromboplastin time (PTT), and clinical hemostasis were evaluated before and after INTERCEPT Plasma transfusion. Baseline and end-of-study serum samples for 33 of 34 patients were tested for potential antibodies to amotosalen- related plasma neoantigens. **Results:** 34 patients received 107 transfusions of INTERCEPT Plasma for factor kinetic studies or therapeutic indications (mean dose = 12.8 ± 8.5 mL/kg). Incremental factor recoveries ranged from 0.9 to 2.4 IU/dL/U/kg and were consistent with factor recovery reference ranges reported in the literature for untreated plasma. Thirteen patients required 77 transfusions for therapeutic indications. There was a statistically significant reduction in PT and PTT observed following INTERCEPT Plasma transfusion (Table 1). INTERCEPT Plasma provided effective hemostasis and was well tolerated. No antibodies to amotosalen-related neoantigens were detected.

Table 1. Response of the PT and PTT to Transfusions of INTERCEPT Plasma (mean + SD)

Patient Group		Pre-Transfsion (secs)	Post-Transfusion (secs)	p-value
All Transfusions	PT	20.7 ±22.2	13.8 ±2.4	<0.001
	PTT	51.2 ±29.3	32.0 ±5.1	<0.001

Conclusions: INTERCEPT Plasma transfusion exhibited factor kinetics and therapeutic efficacy consistent with untreated plasma in patients with congenital coagulation factor deficiencies.

PS4: Immunohematology

PS401

Screening for Antibodies to Low Frequent Red Cell Antigens in Blood Donors

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Purpose: Antibodies to low frequent red cell antigens are often not detected because of the lack of appropriate antigen positive test cells in antibody screening and identification panels. After a severe hemolytic transfusion reaction caused by anti-Wr(a) in a patient with stomach cancer this study evaluates the rate of antibodies against several low frequent antigens in blood donors in southwestern Germany. **Methods:** Routine antibody screening test was performed in 11.313 blood donors (5.009 female and 6.304 male donors) using a solid phase antiglobulin test with a 2-cell panel (Solidscreen II, Biotest, Germany) and 4 additional test cells carrying the following low frequent antigens: Wr(a), V, Vs, Vw, Lu(a), Kp(a), Co(b). The antibody specificity of the positive samples was determined in the gel test method (DiaMed ID, LISS-Coombs) using a 22-cell identification panel including 2 different positive test cells for each investigated low frequent antigen. **Results**: The following 249 red cell antibodies were found (please refer to the table below). The most frequent ones were anti-Vw and anti Wr(a). They represented 80% of all detected antibodies. The rate of anti-Vw was 1,08% in man and 0,88% in women (1,2 times higher in men than in women). The rate of anti-Wr(a) was 0,52% in men and 1,06% in women (2,0 times higher in women than in men). No anti-V, anti-Vs, anti-Lu(a), anti-Kp(a) and anti-Co(b) could be found. **Conclusions:** Anti-Vw and anti-Wr(a) are the most common red cell antibodies in blood donors in southwestern Germany. They are more frequent than the sum of all other detected antibodies. Each of them is about 10 times more frequent than anti-D. As they are clinically significant, they are probably the most dangerous antibodies when using type and screen in pretransfusion management only. Antibodies to the other investigated low frequent antigens are not common.

PS402

A Particle Gel Immuno-Assay (PaGIA) for Detection of IgA-Deficiency and Antibodies to IgA

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Purpose: Antibodies to IgA are frequently found in patients with selective IgA deficiency, and in patients with common variable immunodeficiency. Anti-IgA may lead to severe transfusion reactions. The establishment of a standard test for a simultaneous detection of IgA deficiency and antibodies to IgA may help to prevent anaphylactic transfusion reactions in affected patients. **Methods***:* Coloured polystyrene beads were coated with either human IgA or rabbit anti-human IgA. The beads were incubated with patient's plasma (5 min) and centrifuged (10 min) through a gel filtration matrix (Particle gel immuno assay, ID-PaGIA, DiaMed AG, Cressier sur Morat, Switzerland). Agglutinated particles were trapped on top of the gel or dispersed within the gel (positive reaction), and non-agglutinated particles were pelleted at the bottom of the microtube (negative reaction). **Results***:* The preparated test components were stable for more than six months. 26 of 28 sera from patients with less than 10 mg IgA per dl were confirmed to be IgA-deficient by the new test. The remaining 2 serum samples contained roughly 10 mg per dl and gave positive reactions. All 10 patients with more than 10 mg IgA per dl showed positive reactions by the IgA PaGIA. The anti-IgA PaGIA was positive in 23 of 32 sera suspected to contain anti-IgA, and the anti-IgA ELISA was positive in 24 cases. Seven sera were negative in both tests. **Conclusion***:* The IgA-deficiency PaGIA helps to identify patients with IgA deficiency, and the anti-IgA PaGIA allows the detection of anti-IgA. The test is rapidly performed, and the results can be read visually with ease.

PS403

Defined influence of various immunoglobulin preparations on red blood cell - endothelial cell interactions and on consecutive endothelial cell gene expression

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Purpose: Under certain conditions, endothelial cells have the capacity to act as antigen presenting cells. Close interactions occur between human microvascular endothelial cells (EC) and red blood cells (RBC) under physiological conditions. We aimed at setting up an experimental system allowing for the analysis of the functional impact of normal plasma and of anti-RBC allo- and autoantibodies on the RBC-EC interaction and on the consecutive endothelial cell gene expression. Such experimental system might help to characterise the role of endothelial cells for mechanisms of anti-RBC immunity, and - in addition - serve to set up functional quality markers for cellular blood preparations. **Methods:** We established protocols to investigate RBC-EC interactions under real-time conditions using optical biosensor technology (IAsys, Thermo Labsystems, Cambridge, UK). Interactions were performed in the presence of HBS and various plasma preparations (i.e. normal plasma, plasma enriched in anti-RBC-alloantibodies, warm reactive autoantibodies or cold reactive autoantibodies, normal human IgG for therapeutic intravenous use, IgM purified from an IgM enriched therapeutic preparation

of normal human immunoglobulin). Endothelial cell gene expression profiles were analysed using PIQOR™ Immunology Microarrays (Memorec Biotec GmbH, Cologne, Germany). **Results:** Various antibody preparations involved in the anti-RBC immune response influence RBC-EC interactions specifically, as demonstrated by optical biosensor technology, and induce specific gene expression profiles in EC. **Conclusion:** The experimental design of our study principally allows for the identification of several marker genes for RBC-EC interactions that might help to characterize the specific role of anti-RBC allo- and autoantibodies for the RBC-EC interaction, and that might be useful parameters to describe the quality of cellular blood preparations at the functional level.

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PS404

Capillary Centrifugation for Blood Group Serology diagnostics with Distinct Areas for Positive and Negative Reactions

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Purpose: Gel techniques are well established diagnostics in blood group serology. Ease of handling, stable endpoint, and no need for washing steps in the indirect Antiglobulin Test (IAT) are key strengths of these systems. However, weak positive results are sometimes difficult to discern from negatives. The purpose of this study was to develop an agglutination format with distinct areas for positive and negative reactions, which allows for an IAT without washing steps. **Methods:** A plastic chip, similar in size with an ID-Card (DiaMed), containing an intrinsic microcapillary system, was constructed with the following top to bottom design: 1) Reaction chamber; 2) reagent channel with prefilled reagents; 3) capillary system; 4) flash-sized chamber. The capillary zone and the flash-sized chamber are the areas of positive and negative reactions, respectively. Forward Typing: 10 µl of diluted whole blood are pipetted into the reaction chamber of a chip carrying reagent channels filled with anti-A or anti-B. The chip is centrifuged in an ID-Centrifuge. Antibody Screening with IAT: 25 µl of 0.8 % Screening Cells and 10 µl of patient plasma are pipetted into the reaction chamber of a chip containing Coombs reagent. The chip is incubated for 15 minutes at 37 ° C and then centrifuged as above. Positive and negative results are recognized as haemagglutinates that are retained within the capillary system or as a button of red cells in the negative chamber**. Results:** 20 blood samples (including Ax and A3) have been tested in a prototype chip containing anti-A or anti-B reagent. 10 patient plasma containing irregular antibodies against blood group antigens and 10 patient plasma of blood donors without detectable irregular antibodies have been tested in a prototype Coombs chip. All results were in agreement with the results received with similar ID-Cards. Further experiments have demonstrated the feasibility of the new device also for other applications, such as reverse typing and enzyme test. **Conclusions:** The results of this feasibility study indicate that this technique may overcome disadvantages of gel techniques, i.e. ease of interpretation of very weak positive reactions and lot-to-lot variances.

PS405

Difficulties in the Detection of Kidd Antibodies

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Background: Since 1998 we perform the direct antiglobulintest (DAT) as a part of each blood grouping, and an autocontrol test (ACT) in addition to cross-matching. Whenever one of these tests is positive after preceding transfusions or in case of unclear anaemia or haemolysis, eluates are prepared and tested. We report on the determination of Kidd antibodies which have only been detected by investigation of the eluates. **Material/Methods:** EDTA blood samples of patients; blood grouping (ca. 93,000), antibody screening (ca. 156,000), cross-matches (ca. 350,000), DAT, etc. were performed using geltest (ID, Diamed). Acid elution using ELU-Kit (Gamma Biologicals). Eluates were tested by Capture R screening and identification test (Immucor). **Results and Conclusions:** We found 15 Kidd antibodies (13x anti-Jk(a), 2x anti-Jk(b)) by antibody elution from the red cells of the patients' samples which showed either positive ACT (n=14) and/or DAT (n=14). DAT showed coating of the red cells either with IgG (6) or IgG + C3d (3) or C3d (5) alone. Additional testing of the sera with ID-antibody screening test was negative in 12 cases (A) (10x anti-Jk(a), 2x anti-Jk(b)). In 7 of these, the antibodies (5x anti-Jk(a), 2x anti-Jk(b)) could also be shown in the plasma samples with the Capture R which is not routinely used. In five cases the antibodies could only be detected in the eluates by means of Capture R. In five of these 12 patients (A) a follow-up was possible. In four of them (2 with antibodies detectable in the sera by Capture R) the antibody screening with ID-test even remained negative when investigated after a week or later. Similar diagnostic problems have only be observed with anti-c (two cases). In respect to the frequency it may be a rare problem. In regard to possible haemolytic transfusion reactions we recommend to regularly include DAT and ACT into the routine diagnostics for preparation of red cell transfusion, and to investigate the eluates whenever these tests become positive after preceding RCC transfusions with or without haemolysis.

PS406

Rapid Multi-parameter Typing of 10 blood groups with stable end-point

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Purpose: Current rapid tests for blood typing do not have stable end-points. Sophisticated techniques, which provide more objective and stable results, are rather slow and need a centrifugation step. All currently existing blood typing methods have in common that only one parameter at once can be determined. The purpose of this study was to develop a blood grouping format with multi-parameter testing in a single assay, providing for a stable end-point, but without centrifugation. **Methods:** A lateral flow device was designed with a separation membrane equipped in a cassette housing having a central application zone and 2 equidistant detection areas printed with parallel lines of antibody reagents directed against blood groups A, B, AB, D and C, Cw, c, E, e, and K, respectively. Further, both detection areas contain a flow control spot and an auto control spot. 50 µl of EDTA-anticoagulated blood is diluted with 200 µl of a buffer solution. 100 µl of the resulting suspension are pipetted into the application zone, followed by 300 µl of a washing buffer. Results can be read after 5 minutes. Positive results are recognized as distinct red bands, negative results are recognized by the absence of the respective band. In an alternate test format, positive results are shown by a plus (+) symbol and negative results by a minus (-) symbol. **Results:** The bloods of 1415 donors, previously typed for the respective blood groups with the Olympus PK-80, have been tested with the new lateral flow test. The results for all antigens were in agreement with those received from PK-80. **Conclusions:** A simple, rapid and flexible method for blood typing with stable end-point is presented, allowing for: 1) Miniaturisation. 2) Parallel testing of multiple blood groups in the same assay. 3) Use in non-laboratory situations. Further evaluations including reverse typing are currently under way.

PS407

Survey for *RHD* alleles among random D positive donors

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Purpose: Variant D are frequent in some populations, like Africans. Variant *RHD* alleles are considered rare in Europeans. However, this assumption has not been confirmed by a systematic study at the molecular level. **Methods:** We performed a random survey among blood donors in South-Western Germany: 500 samples of Rhesus phenotype ccDee, 250 CcDee and 250 ccDEe. They were tested by PCR-SSP for selected nucleotide variations that are coding for S3C (indicative of weak D type 3); L110P (D cat VII); A149D (weak D type 5); N152T; T201R and F223V (weak D type 4); V270G (weak D type 1); T283I (DHMi); M295I (weak D type 11); G355S (DNB); T379M (DAU-0); and G385A (weak D type 2). All PCR-SSP positive samples were confirmed by sequencing the ten *RHD* exons from genomic DNA. To date, the nucleotide sequence for *RHD* exon 5 has been tested in 700 samples. **Results:** In the ccDee phenotype, 5 samples were positive for weak D type 4.0; 3 samples for weak D type 4.1; 5 samples for DAU-0; 1 sample for

weak D type 11; 1 sample for D_category V type I + DAU; the molecular basis of the latter sample was determined as *RHD*(F223V, E233Q, T379M). There were 2 DNB in the CcDee samples. No variant *RHD* allele was found in the ccDEe samples. Sequencing exon 5 revealed so far one additional new *RHD* allele, *RHD*(R234W), which occurred as a heterozygous compound together with the regular *RHD* allele. **Conclusion:** We performed a systematic study at the molecular level and found variant *RHD* alleles, which are consistent with previous reports. The new allele *RHD*(F223V, E233Q, T379M) is dubbed DAU-5, represents a recombination between two known alleles of the Eurasian and DAU clusters, and thus adds to the understanding of the *RHD* phylogeny. By sequencing *RHD* exon 5 another new *RHD* allele, *RHD*(R234W), was found. Our approach showed that - even within the European population - the variety of *RHD* alleles may be larger than anticipated and biologic relevant information may be gleaned from such systematic studies.

PS408

West-Austrian DNA Polymorphism of ABO alleles causing weak blood group A expression

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Background: The reason for weak expression of A antigens, as found e.g. in A3, Aend, Ax and Aw, were shown to be in part caused by altered A alleles of the ABO gene locus. To investigate the DNA-polymorphism of these alleles in our population, 11 individuals of known weak ABO blood group A expression were investigated serologically and with molecular methods. **Study Design and Methods:** ABO serology was done using gel matrix testing and plate test with human polyclonal anti-A, anti-H and anti-A hel. Blood samples of all serologically predefined "weak As" (without further specification) showed a weak reaction with human polyclonal anti-A and/or anti-AB and practically negative reactions with anti-A hel. Eleven donors with ABO genotype A/O (e.g. presence of an A allele and one allele showing a deletion of G261 in exon 6 of the ABO gene) were selected for DNA sequencing of exons 2 – 7 and intron 6 of the A allele. Discrimination of the A allele long range PCR reverse (exon 2 – 6) and forward (exon 6 – 7) amplification was accomplished by sequence specific priming at G261. **Results:** Of 11 "weak A" alleles, 4 were identical to the DNA sequence of A101 (allele designation at www.bioc.aecom.yu.edu, AF134412) and 1 was identical to A102 (X84746al). Two other A alleles also showed an A101 specific coding sequence, but with an insertion of 2 independent As in intron 6 and a C285G substitution in exon 6, respectively. Another 4 were identical to A101 but with substitutions C467T and G829A and a deletion of C1061; the later 4 were identical to A302 (no accession, Barjas-Castro ML, 2000) beside the additional C467T. **Conclusions:** Since 4(5) out of 11 samples with "weak A" expression showed A alleles identical to A101, other reasons for weak A expression than blood group A allele polymorphism can not be excluded. However, 6(5) showed DNA variations compared to A101, with 4 of them representing a unique allele, comparable to A302. Weak A expression is relatively frequent in the investigated donor population with a minimum (allele)frequency of at least 0,00081 (e.g. frequency of "weakA"/O heterozygous individuals is about 1 among 1000). Therefore, evaluation of new (monoclonal) ABO test sera is of importance and could be optimized using a molecularly predefined panel of known "weak A" test cells.

PS409

An alternative translation of a variant *ABO*A* allele caused by a translation-initiator mutation leads to a weak A phenotype

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Background: Until now, weak blood group A and B phenotypes have been correlated with single base substitutions occurring in the coding and non-coding sequences from exons 2 to 7 of the *ABO* gene. The resulting A or B glycosyltransferases are predicted to exhibit single amino acid changes and/or truncated or elongated C-termini, affecting the proteins' enzyme activities by altering the sugar binding site. **Methods:** An healthy blood donor from Germany diagnosed as having weak A antigen expression and relatives of him were subjected to extended ABO typing. Serologic investigations were performed using standard techniques and flow cytometry. The genetic basis of the ABO phenotypes was determined by PCR-SSP and subsequent sequence analysis of the complete sequence and two regulatory regions of the *ABO* gene. HeLa cells were used to transfect ABO expression plasmids. **Results:** The donor's red blood cells were typed as $A_{weak}B$ and his serum contained weakly reactive anti-A_1 antibodies. Sequence analysis identified a single T→C transition in the +2 position of the ATG codon for translation initiation on a *ABO*A101*/*ABO*B101* background. This new point mutation resulted in amino acid exchange from methionine to threonine at position 1. ABO typing of the donor's children revealed the phenotype A_1B and the genotype *ABO*A101*/*ABO*B101* for the son and the phenotype A_1 and the genotype *ABO*A101*/*ABO*Avar* for the daughter, demonstrating that the new polymorphism is located in an *ABO*A* allele. In the transfection studies, no decrease of A activity was observed on HeLa cells transfected with plasmids containing the variant *ABO*A* allele. However, a significant reduction of A antigen expression (36-70 %) was detectable on HeLa cells co-transfected with plasmids specific for the variant *ABO*A* allele and the normal *ABO*B101* allele compared to cells co-transfected with *ABO*A101* and *ABO*B101* constructs. A normal A activity was observed with an *ABO*A101* construct where the codons encoding for Met1 and a potential start site at amino acid position 26 were artificially disrupted. **Conclusion:** The data provide evidence that a nearly full functional A transferase can be produced by alternative translation start sites in the transmembrane domain or stem region. The weak blood group A phenotype of the donor most likely resulted from competition between a normal B transferase and a N-truncated A transferase.

PS410

Weak D type 1.1 confounds weak D typing by PCR

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Background: The molecular classification of weak D allowed to develop transfusion strategies for specific weak D types. For example, no allo-anti-D immunization has been observed in individuals carrying the frequent weak D types type 1 to type 3, and a D positive transfusion strategy is generally advocated if such weak D type is detected. The classification of weak D types is often accomplished by PCR, checking only the presence of the characteristic mutation. The validity of this approach depends on the absence of variants of the common weak D types carrying additional mutations that might alter their phenotype and clinical characteristics. **Methods:** For blood donor typing, samples with known "weak D" phenotype (including known partial D) were re-checked in a Gel card system (DiaMed AB0/D). This card includes two anti-D reactions (polyclonal anti-D and monoclonal anti-D) that give a ++ to +++ reactivity with samples of frequent weak D types. Samples with unusual phenotypes (e.g. reactivity only 0 to +) were further investigated by *RHD* PCR, weak D PCR or sequencing. **Results:** A sample that was negative with both anti-D of the DiaMed AB0/D card was scheduled for molecular typing. Surprisingly, the sample was molecularly classified as weak D type 1. Since this molecular result was considered incompatible with the phenotype, the full *RHD* coding sequence was established by sequencing of genomic DNA. An *RHD*(L18V, V270G) allele dubbed weak D type 1.1 was detected. In subsequent analyses, this allele was detected in two additional samples from Northern Germany. **Conclusion:** In Northern Germany, weak D type 1.1 is prevalent among weak D samples with very weak D expression. Typing of weak D samples should consider serology to avoid mistyping of alleles like *RHD*(L18V, V270G) as frequent weak D types by PCR. A typing strategy exclusively based on weak D PCR may result in erroneous phenotype predictions and, possibly, inappropriate transfusion strategies.

PS411

Missense mutations outside the catalytic domain of the ABO glycosyltransferase can cause weak blood group A and B phenotypes. In vitro mutagenesis study

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Background: Weak blood group A and B phenotypes have been correlated with single base substitutions predominantly occurring in exons 6 and 7, which encode for the catalytic domain of the ABO glycosyltransferase. Only less is known about the impact of missense mutations upstream of exon 6 on the activity of A and B transferases. **Methods:** A panel of ABO expression plasmids was established containing the six known missense mutations of the coding sequence from exon 2 to 5 of the *ABO* gene. Among them were the rare mutations 46G>A, 190G>A and 203G>C and the common mutations 106G>T, 188G>A, and 220C>T which are characteristic for the *ABO*O02* allele. Blood group A-specific plasmids with single or multiple mutations were constructed by site directed mutagenesis. HeLa cells were used to transfect ABO expression plasmids. Plasmids encoding for the blood groups A_1, B and O as well as for defined A and B subgroups were used as controls. A and B antigen expression on transfectants was analyzed by flow cytometry using monoclonal antibodies. **Results:** Transfection of plasmids containing single missense mutations or combinations of them resulted in a decrease of A antigen expressing cells (AEC) in a range from 2 to 13 % and of mean fluorescence intensity (MFI) in a range from 2 to 30 % compared to HeLa cells transfected with the *ABO*A101* control construct. A reduction of A activity in a range from 4 to 31% (AEC) and from 8 to 40% (MFI) was detectable on HeLa cells transfected with both a variant *ABO*A* allele and the normal *ABO*B101* allele compared to cells co-transfected with *ABO*A101* and *ABO*B101* constructs. In the co-expression experiments, the lowest expression levels were comparable with the A activity of HeLa cells transfected with a control plasmid containing a blood group A_x-related mutation. The weakest values for A antigen expression were observed for plasmids with mutations causing amino acid changes in the stem region of the A transferase. **Conclusion:** Mutations in early exons, outside the enzyme's active site, can affect expression of ABO blood group antigens. In particular, in AB phenotypes weak A and B subgroups can arise from competition between normal A or B glycosyltransferases and variant forms that contain amino acid changes in the transmembranous domain and stem region.

PS412

Frequency of DAU, RHD psi and Cdes in South African blood donors

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Purpose: To evaluate a new PCR-SSP system with prepipetted ready-to-use reagents for the prediction of the D, C, c, E and e phenotype from DNA in individuals from African descent and the detection of variant *RHD* alleles. **Methods:** In a South African donation centre we obtained anticoagulated whole blood from 439 Whites, 177 Asians, 53 Blacks and 45 individuals of mixed race, referred to as "Coloured" in South Africa. These samples were tested for ABO and D, stored at 4°C and send to Germany where we determined the complete Rh formula with standard serological methods (gel centrifugation test). We used an automated protocol for DNA extraction (Agowa, Berlin, Germany) and tested with a multiplex PCR containing primers for *RHDψ*, *RHD* intron 7 and *RHD*(W16X). Additionally, we tested for variant *RHD* alleles and the *C, c E* and *e* alleles of *RHCE* with SSP kits (RH-TYPE, Partial-D TYPE, BAG, Lich, Germany) in Black and Coloured donors, only. **Results:** The percentage of D-negative individuals was 19.4 in Whites, 2.8 in Asians, 1.9 in Blacks and 2.2 in Coloured. *RHDψ* was not found in Whites and Asians, 5 (9.4%) Blacks tested positive for *RHDψ* (all *RHD*/*RHDψ*), 1 (2.2%) Coloured individual carried the *RHDψ* allele (D-negative, homo- or hemizygous). The complete Rh phenotype and genotype was concordant in 97 of 98 individuals tested. In one C- sample we obtained a positive result with the *Cde*^{s} SSP. Using published primers (Tax MGHM et al. Transfusion 2002; 42:634) for the *Cde*^{s} hybrid exon 3 the presence of the *Cde*^{s} allele was confirmed. The *Cde*^{s} allele was found in 3 of 53 (5.7%) Blacks and 1 of 45 (2.2%) Coloured donors. The variant *RHD* allele *DAU* was observed in 8 Black and 2 Coloured donors. **Conclusion:** In this study we add data to the published literature with respect to the frequency of *RHDψ*, *Cde*^{s} and *DAU*. The prediction of the D, c, E and e phenotype from DNA with SSP seems to be reliable in Black and Coloured South African blood donors. Since two independent SSP methods indicated the presence of the *Cde*^{s} allele in a donor who tested C-negative with stored red cells, a repeat serological test is required in order to interpret this single discrepancy in this study.

PS413

New polymorphic sites in *RHCE* exon 5 found in a patient with weak RH-Ce expression

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Purpose: Variants of the RhC or Rhe antigens have been described previously, showing that the requirements for expression of these antigens are not fully understood. Here we present data for a new *RHCE*-allele expressing a weak rhesus e antigen in a caucasoid person. **Methods:** Serological testing has been done with mono- and polyclonal anti-C (P3x25513 G8/392; MS 273; F388F3; MS 24) and –e (267; M16; MS 21; MS 63;e1-Y-B4K; MS62/69) sera (Biotest, Biolith & Ortho). *RHD* and *RHCE* typing was performed by multiplex-PCR with 24 fluorescent primer pairs. Positive results were obtained for *RHD*-exons 2-7, 9, 10 and polymorphisms associated with antigens C, c, Cw, E and e. Sequencing of *RHCE*-sequences, including exons 1 to 10 and intron/exon borders, were done by direct taq cycle-sequencing using bigdye-terminators v.1.1 in an ABI310 (Applied Biosystems). **Results:** A. Serology: anti-C (polyclonal, Biolith and Ortho) very weak; ID-micro typing: weak; anti-e, monclonal sera: negative (Seraclone) and very weak (Ortho); polyclonal sera: negative (Optima); ID-micro typing: weak. B. DNA: After sequencing *RHCE* exons 1 to 10, three differences compared to the wild type sequence were found at codons 223 (G667T / transmembrane region 7), 233 (G697C / loop 4), and 238 (G712A / loop 4). G667T lies in close proximity to Ala226 (the e-antigen polymorphism). These findings represent an extension of variant-e ceMO (Noizat-Pirenne et al., BJH 2001) including the polymorphic sites 667, but not 697 and 712. In contrast, the E III variant (Noizat-Pirenne et al., 1996) has been shown to harbour the polymorphisms 697 and 712, but not 667, adjacent to cE. **Conclusion:** The substitution of the *RHCE*-specific nucleotides 667, 697, 712 (combined with 676 for e) with their *RHD* specific counterparts leads to an altered and weakened expression of the Rh e antigen (variant e). Since two amino acid changes lie in extracellular loops of the Ce antigen, it has to be suggested that this altered e-antigen may be involved in allo-immunisation.

PS414

Four new RHD-alleles with previously unknown polymorphisms detected in RHD-exons 4, 5 and 9

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Purpose: In order to prevent immunization with D-antigen in D-negative individuals every blood donor is screened for the absence or appearance of complete or partial D-epitopes on the rbc surface with several D-specific sera. We analysed blood samples in a first step with two mAbs (BS226, BS232) and IgM-/ IgG- specific "blend" sera (Biotest). Second, chromosomal DNA was extracted from EDTA-anticoagulated blood samples for sequence analysis. While genotyping about 260 samples with serologic suspect for weak D with multiplex-PCR methods and sequencing, we found four weak-D samples that could not be classified according to the weak D RHD-nomenclature. Here we present data of four new RHD alleles with so far unpublished polymorphisms.
Methods: Total DNA was amplified with fluorogeneic primers (a) to detect RHD exons 2-7, 9 & 10; (b) polymorphisms known for D-weaks (type -1, -2,

-3, -4, -5), partial D´s (D-VII, D-HMi) and RHCE (C, c, Cw, E, e). Amplicons were screened using the Genescan method in an ABI 310 (Applied Biosystems); (d) sequencing of RHD-sequences, including exons 1 to 10 and all intron/exon borders, were performed with taq cycle-sequencing kits and big dye - terminators (v.1.1).

Results and Conclusion: PM=polymorphism; range of serologic results: 0 → 4+;

(1=BS226, 20°C; 2=BS232, 20°C; 3=blend sera, 20°C; 4=blend sera-IAT, 37°C):

Allele-A: exon 4 (C513A); serologic data not available

Allele-B: exon 4 (T521A), serologic data not available

Allele-C: exon 9 (C1221A); SER (1:--/2:--) (3:-- / 4: +)

Allele-D: exon 5 (684del3); deltion of three nucleotides (GAG); SER (1:--/2:--) (3:--/ 4: +++)

The deletion in allele D affects the codons 228 (CTG) and 229 (AGA) with loss of a single amino acid (del229Arg) in extracellular loop 4, representing a weakened D phenotype.

All of these alleles were tested negative for irregular anti-D antibodies. Frequencies could not be estimated. Weak-D data were reported to the Rhesus database (www.uni-ulm.de/~fwagner/RH/RB/).

PS415

Non-deletional *ABO*O* alleles express weak blood group A phenotypes

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Background: Due to a single-base deletion, the vast majority of *ABO*O* alleles encode for a truncated and catalytically inactive ABO glycosyltransferase, leading to the generation of a premature stop codon. Less frequent non-deletional *ABO*O* alleles such as *ABO*O03*, on the other hand, have non-synonymous mutations that may abolish the protein's enzyme activity by altering its sugar-binding site. **Methods:** In the present study, we performed extensive ABO phenotyping and genotyping in healthy blood group O donors with weak anti-A isoagglutinins and their relatives as well as in blood group O donors selected for the presence of *ABO*O03*. HeLa cells were used to transfect ABO expression plasmids. **Results:** Donors and relatives found to be homozygous (n=2) or heterozygous (n=14) for *ABO*O03* and the rare *ABO*O03*-like allele *ABO*Aw08*, respectively, showed weak A antigen expression detectable only by adsorption-elution (n=15) or by monoclonal anti-A typing (n=1). The serum of most donors (n=13) contained weak anti-A; in the remaining donors, anti-A isoagglutinin reactivity was in the normal range. In the transfection studies, weak A antigen expression on HeLa cells transfected with plasmids containing *ABO*O03* or *ABO*Aw08* expression constructs was detectable only by adsorption-elution. **Conclusion:** The data provide evidence that non-deletional *ABO*O03-like* alleles produce detectable amounts of A antigens.

PL2: Future Perspectives in Cellular Therapy and in Transfusion Medicine

PL201

Mesenchymal Cells and Hematopoietic Stem Cell Transplantation

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The bone marrow serves as a reservoir for different classes of stem cells. In addition to hematopoietic stem cells the bone marrow comprises a population of marrow stromal cells or mesenchymal cells. These exhibit multilineage differentiation capacity and are able to generate progenitors with restricted developmental potential, including fibroblast, osteoblast, adipocytes and chondrocyte progenitors. MSCs might be used either to replace host cells in a marrow microenvironment that have damaged by chemotherapy or irradiation, or as vehicles for gene therapy.

Although the bone marrow serves as a primary reservoir for MSCs, their presence has been reported in a variety of other tissues, including periosteum and muscle connective tissue, fetal bone marrow, liver and blood. MSCs have identified in fetal blood and by some laboratories also in umbilical cord blood, the frequency likely being extremely low. Recently, we have isolated MSCs from human amniotic fluid. A quantity of several ml of second trimester amniotic fluid was sufficient to culture and expand these cells to the numbers required for cotransplantation in an adult recipient.

Several studies suggest that MSCs may play a role in modulation of immune responses. MSCs are poor antigen presenting cells and do not express MHC class II or costimulatory molecules. In accordance, expanded MSCs do not stimulate T cell proliferation in mixed lymphocyte reactions (MLR) and are able to down regulate alloreactive T cell responses when added to mixed lymphocyte cultures.

Several reports indicate that cotransplantation of MSCs and hematopoietic stem cells may enhance hematopoietic engraftment by mechanisms that are still unclear. These observations form the basis for clinical studies with bone marrow-derived expanded MSCs in an attempt to modulate immune responses and accelerate hematopoietic reconstitution. In a multicenter phase I/II study, allogeneic donor bone marrow-derived MSCs were co-infused in patients with hematological malignancies undergoing matched sibling transplantation. A matched pair analysis suggests a reduced incidence of acute and chronic Graft-Versus-Host Disease. These results warrant further clinical phase III trials in order to determine the efficacy of this approach. We are currently working on a common European MSC expansion protocol under GMP conditions, to facilitate collaborative clinical studies.

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PL202

Adult stem cells and their potential in the treatment of cardiovascular diseases

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Recent data suggest that adult stem cells retain a degree of developmental plasticity. Bone marrow stem cells (BMSC) of adult mice are being widely studied for their potential to generate nonhematopoietic cell types. We have investigated the potential of BMSC to regenerate acutely infarcted myocardium in several animal models. Infarctions were produced in the left ventricle (LV) of adult female mice by ligation of the left coronary artery (LCA). The infarcts were treated with Lin^- $c\text{-}kit^+$ bone marrow cells. Myocyte-specific proteins, including several transcription factors and connexin 43, were detected in the developing cardiomyocytes and LV function was improved. In a second series of experiments we investigated the ability of murine BMSC mobilized by 5 daily injections of stem cell factor and granulocyte colony-stimulating factor to traffic to acutely infarcted myocardium and promote regeneration. At 27 days after cytokine treatment and LCA occlusion the cytokine-mobilized BMSC had regenerated a new band of myocardium in the left ventricle. Partial repair of the infarcted myocardium resulted in improved heart function and greater survival. This repair did not appear to be the result of stem cell-cardiomyocyte fusion since the average volume of the new cardiomyocytes was one third of that of mature cardiomyocytes. Also, the number of new cardiomyocytes was an order of magnitude greater than the mature cells that they replaced. A brief 1 to 3 hour exposure to ischemia resulting from LCA occlusion is believed to lead to irreversible damage of the cardiomyocytes distal to the LCA occlusion, hence fusion, in this setting, would involve apoptotic cardiomyocytes. We have also tested whether cytokine treatment might be an effective therapy stimulating myocardial repair in a rhesus monkey model of acutely induced myocardial infarction. MRI analysis of gadolinium uptake by cardiomyocytes was performed 1, 6 and 12 weeks after ligation of the anterior descending branch of the LCA to determine infarct size. MRI indices showed no variation in infarct size in the different cytokine treatment groups and control animals. LV ejection fraction was not improved. Microscopic examination of hearts at 12 weeks post-myocardial infarction indicated extensive collagen deposition and scar formation in the LV with no clear evidence for regeneration in the infarct zone in any group. Regeneration of acute myocardial infarctions has been studied in several clinical trials using autologous bone marrow cells. Cells were transplanted via the infarct related coronary artery or injected directly into the myocardium after angioplasty, stent implantation or coronary artery bypass grafting. Patients were followed for 3 to 9 months. Although the trials differ to some degree in their clinical findings, all report partial improvement in cardiac reperfusion within the zone of infarction and/or some improvement in hemodynamic functions. These trials demonstrate short-term safety and feasibility but do not conclusively established efficacy. The potential benefit and the need for additional testing in the use of stem cells for myocardial repair will be discussed.

PL203

The Changing Landscape of Transfusion Medicine: 2004–2015

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Born at the dawn of the 20^{th} Century, Transfusion Medicine has seen tremendous progress and technical achievement during the last 100 years. As the pace of change increases, the prospects for the future are even brighter. This lecture will review the recent past developments and provide conjecture on future advances in each of the following areas: blood donor services, infectious hazards, non-infectious hazards, process risk, diagnostics and therapeutics. Emerging technologies such as gene array and radio-frequency identification are likely to produce advances in the safety of blood transfusion therapy. Nanotechnology may re-invent the indications for blood therapies. The field of new therapeutics should be the fastest growing area and the lecture will highlight recent advances in drug development and molecular therapeutics. We highlight the promise of therapeutic cloning and nuclear

transfer and its impact on cellular therapies and stem cell technology. The lecture concludes with an impassioned reminder of the real challenges that await us.

Thursday, September 23, 2004: Main Sessions

SP7: Safety Aspects of Hemotherapy II – Bacterial Detection

SP701

Testing Strategies for Bacteria

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Bacterial transfusion transmission remains a significant problem in transfusion medicine. In the UK the Serious Hazards of Transfusion (SHOT) surveillance scheme registered, between 1995 and 2003, 29 incidents due to bacterial contamination, seven of which were fatal. Platelet concentrates are responsible for the majority of bacterial transmissions due to the 22ºC storage temperature of the product. SHOT reports that 25 of the 29 bacterial transmissions (86%) were due to platelet concentrates. The interventions of diversion and improved donor arm disinfection will significantly reduce bacterial contamination of blood components. These interventions though do not remove the necessity for the screening of blood components and will not prevent transmission from a bacteraemic donor. Blood services in Belgium, Netherlands, Wales and the USA have introduced mandatory screening of all platelet concentrates. The systems currently available are the BacT/ALERT automated microbial detection system, Pall enhanced Bacterial Detection System (eBDS) and the Scansystem. BacT/ALERT and the Pall eBDS are sensitive culture systems. Detection times for BacT/ALERT are in the order of 18-24 hours. The Pall eBDS requires a minimum incubation period of 24 hours. In contrast the Scansystem which uses fluorescent cytometric technology produces a result in under 90 minutes. Sensitivity of the Bact/ALERT and Pall eBDS are in the order of 0.1 cfu/ml and the Scansystem 1000 cfu/ml. Issues that need to be addressed for the bacterial screening of platelet concentrate are when to test, what size of sample to take and which assay to use. All these issues are related to the fact that unlike viruses, bacteria proliferate in the platelet concentrate bag. The screening of platelet concentrates immediately after preparation would require a sensitive assay and the sample size may need to be large to detect a bacterially contaminated unit, which may be low in bacterial number at this point in time. Culture systems such as the BacT/ALERT and Pall eBDS meet this requirement. Screening platelet concentrates at day two or later in shelf life allows for a possible reduction in sample volume, the possibility of pooling samples and the extension of shelf life to seven days. Culture and a rapid test such as the Scansystem may be used for screening of products at day two or later in shelf life. Indeed, the complex issue of bacterial screening may require a paradigm shift, with regard to the issues of when to test, the number of tests performed and the duration of the validity of a negative result. Bacterial screening will improve blood safety and have a far greater cost benefit than nucleic acid testing (NAT) and leucodepletion.

SP704

Wachstumkinetik von Bakterien in Blutkomponenten

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Hintergrund: Während sich das transfusionsmedizinische Interesse in den letzten Jahrzehnten vorwiegend auf das virale Infektionsrisiko fokussierte, rückt gegenwärtig das bakteriologische Infektionsrisiko in den Mittelpunkt der Transfusionsmedizin. Nach Einführung von molekularbiologischen Screeningmethoden (z. B. Polymerasekettenreaktion) ist das Restrisiko für HCV und HIV Infektionen kleiner als 1:23 Millionen (für die DRK-Blutspendedienste) und damit nahezu Null zu setzen. Dagegen wird das Risiko für bakterielle Infektionen durch Thrombozytenkonzentrate auf 1:2000 bis 1:3000 angegeben. **Material/ Methoden:** Der Nachweis von Bakterien erfolgt in den DRK-Blutspendediensten zur Zeit mit einer automatischen Blutkulturmethode (BacT/Alert) im Rahmen der Qualitätskontrolle. Die Messung dauert dabei bis zu 7 Tagen. Daneben sind Methoden entwickelt worden, die Bakterien innerhalb weniger Stunden messen können. In der vorliegenden Studie wurden Pool-Thrombozytenkonzentrate mit 6 transfusionsmedizinisch relevante Bakterien, in einer Konzentration von 10 CFU/ml, gespikt und anschließend nach 24h, 30h und 36h mit dem Scansystem gemessen. Parallel dazu wurde die jeweilige Keimzahl durch Ausplattieren bestimmt. Zum Ausschluss von Kontaminationen wurden die TK-Beutel mit Hilfe des DNA-Fingerprints, der Gram-Färbung und mikrobiologischer Differenzierung auf Identität mit dem Kontaminationskeim geprüft. Bei den getesteten Bakterien handelt es sich um Isolate aus Blutkomponenten. Es wurden hauptsächlich Bakterienstämme verwendet, deren Fähigkeit zum Wachstum in TK zuvor getestet worden war (PEI-Bakterien-Standards). **Ergebnisse:** *Klebsiella pneumoniae* (PEI-B-08-07), *Escherichia coli* (ATCC 25922) und *Staphylokokkus auerus* (PEI-B-23-03) konnten in allen gespikten Beuteln nach 24h mit Scansystem nachgewiesen werden; *Staphylokokkus epidermidis* (PEI-B-06-05), *Streptokokkus pyogenes* (PEI-B-20-03) konnte zum Teil nach 24h und zu 100% nach 30h mit Scansystem nachgewiesen werden, wohingegen *Propionibakterium acnes* (PEI-B-22-03) bis zum Zeitpunkt 36h zwar auf Blutagarplatten unter anaeroben Bedingungen gewachsen war, jedoch mit dem Scansystem nicht detektiert wurde. Die Fingerprintuntersuchungen ergaben eine 100% Übereinstimmung zwischen den gespikten und im TK-Beutel vorhandenen Bakterien. **Zusammenfassung:** Mit Hilfe der Scansystem Methode können transfusionsmedizinisch relevante Bakterien in Pool-Thrombozytenkonzentraten in Additivlösung zwischen 24h und 30h nach dem Spiken nachgewiesen werden. Eine weitere Studie die mehrere schnelle Bakteriennachweismethoden (FACS, PCR und Scansystem) miteinander bezüglich Sensitivität und Praktikabilität vergleicht, befindet sich in der Planung.

SP8: Adoptive Immotherapy – Immunovaccination

SP801

Immunoregulation by dendritic cells: update on current strategies

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Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play crucial roles as initiators and modulators of adaptive immune responses. DC-based vaccines have been employed to generate T-cell activity against tumor and infectious antigens (Ags). Within recent few years, evidence has accumulated that DCs also have potent capabilities to tolerize T cells in an Ag-specific manner. DCs cultured in the laboratory can suppress auto- or alloimmunity. Current and prospective strategies to promote this inherent immunoregulatory potential of DCs might prove to be important for the therapy of transplant rejection and autoimmune diseases.

SP802

Adoptive immunotherapy using cellular effectors

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The immune system plays a major role in the progression of malignant diseases and the defense of viral infection. Thus, immunotherapy with cytotoxic cells could represent an alternative therapy that is not cross-reactive with previous treatments administered to patients (chemotherapy, radiation therapy). The adoptive transfer of cytotoxic T-lymphocyte or natural killer (NK) cell populations in terms of passive immunotherapy may be able to

support the immune system of immuno-incompetent patients that fail to elicit a vigorous immune response upon active immune strategies, such as e.g. dendritic cell based vaccination. This is true for both, life threatening viral infections after bone marrow transplantation and some cancers that appear to be sensitive to immunotherapy and particularly to the lytic activity of activated natural killer (NK) cells. Moreover, recent advances in the isolation of virus specific T cells (e.g. MHC-Tetramers) or the ability to graft T- and NK cells with the specificity to kill certain tumors using recombinant chimeric antigen or T cell receptors, hold the promise of tailored cellular therapies that will ultimately ad to current treatment options. While these new approaches have proven to be effective in preclinical settings, they demand a revisited analysis of cellular effectors for adoptive immunotherapy in malignancies and viral infection.

SP803

MHC-Streptamer-guided purification of CD8$^+$ CMV-specific T cells for adoptive immunotherapy after hematopoetic stem cell transplantation

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One of the main causes of morbidity and mortality in patients undergoing allogeneic hematopoetic stem cell transplantation (HSCT) is infection or reactivation of human cytomegalovirus (HCMV). Especially the increasing number of HCMV isolates resistant to gancyclovir after HSCT and the toxicity of antiviral treatment explain the urgent search for alternative therapies. Transfer of donor-derived CMV-specific T-cells has proven to be a very effective treatment of CMV infection in HSCT patients. However, the time-, labor-, and cost-extensive procedures required for generation of CMV-specific T cell cultures limited this approach until now to only a few clinical studies performed on low numbers of patients. Here we present a new strategy to purify CMV-specific CD8$^+$ T-cells from donor PBMCs for direct adoptive transfer (without the need of *in vitro* T cell culture) using a novel reversible MHC-Multimer technology, called "MHC Streptamers". We are currently transferring the Strepamer technology to purification-protocols based on paramagnetic beads, in order to allow large scale T cell purification for clinical applications. A first clinical study (supported by Stage Pharmaceuticals GmbH) will be initiated using MHC Streptamers for selection of CMV-specific T-cells.

SP804

Sanglifehrin A, a new selective in vivo inhibitor of il-12 production and endocytosis of dendritic cells

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Purpose: Recently, we have discovered, that the novel cyclophilin-binding immunosuppressant Sanglifehrin A (SFA) blocks bioactive IL-12 production by human dendritic cells (DCs) in vitro. The molecular mechanism of SFA is currently unknown. Here, we have analysed the capacity of SFA to impact on proinflammatory (IL-12p70, TNFα) and immunomodulatory (IL-10) cytokine production and DC endocytosis in vivo. By using independent in vivo models that employ different IL-12 inducers we provide evidence that SFA abrogates IL-12p70 production in vivo while having minor or no effects on IL-10 and TNF-α production. Furthermore, we show, that SFA decreases in vivo antigen uptake (endocytosis) by DCs. **Methods:** Mice (C57BL/10, H2K^b) were injected intraperitoneally (i.p.) for 3 to 10 days with SFA (10 mg/kg/d) or drug vehicle to study drug effects under steady-state conditions. Additionally, to explore SFA's effects specifically on DCs under dynamic conditions, we injected the DC growth factor Flt3L (10 μg/d) and SFA (10 mg/kg/d). Subsequently animals were stimulated with CpG DNA or LPS and IL-4 or injected with FITC-Dextran or FITC-Albumin i.p. for in vivo endocytosis. Four hours later, peripheral blood was drawn and splenic and bone marrow DC subsets were analysed by flow cytometry. **Results:** The data show that a 3-day course of SFA inhibited 70% of in vivo IL-12p70 production, induced by either CpG or LPS/IL-4 stimulation. Under dynamic conditions, a 10-day course of SFA blocked 95% of LPS/IL-4 induced IL-12p70 and 98% of CPG-induced IL-12p70 production in vivo. These effects are not due to suppressive effects of SFA on total DC numbers or DC subsets, as indicated by four colour flow cytometry. The production of TNF-α and IL-10 were only moderately affected by SFA. Our in vivo endocytosis experiments revealed a 75% decrease of antigen uptake. **Conclusion:** We propose that SFA represents functionally a novel class of immunophilin-binding immunosuppressants that has a high selectivity and potency to abrogate production of the major proinflammatory and Th1-skewing cytokine IL-12p70. Furthermore it seems to have a profound impact on antigen presentation, one of the pivotal functions of dendritic cells.

SP805

Current report from the Study Group of the German Society for Blood Transfusion and Immunohaematology (DGTI) on the use of G-CSF in granulocyte donors

*U.J.H. Sachs and J. Bux on behalf of the study group**
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The aim of this trial is to study the safety and efficacy of granulocyte colony-stimulating factor (G-CSF) in the conditioning of granulocyte donors. Eighteen German blood banks have adopted a common protocol on G-CSF stimulation, preparation of granulocytes, reporting of adverse effects of both, G-CSF stimulation and apheresis procedure, and follow up of donors over a 2-years period. Data on approximately 1.000 granulocyte donations from 300 donors will be presented. Main outcomes of our study are: No severe G-CSF side-effects were observed. The mean granulocyte yield was approx. 4 x 10^{10} per apheresis. Mild thrombocytopenia and anemia were occasionally observed after granulocyte donation, but all side effects were completely reversible. A high number of donors expressed their willingness to donate granulocytes again. No adverse events were reported from donors at the follow-up 2 years after donation. Transfusion of granulocytes was well tolerated with only minor adverse reactions in the recipients. In conclusion, these data demonstrate that G-CSF stimulation as well as transfusion of G-CSF mobilized granulocytes is well tolerated by donors and recipients, respectively. In consequence, we should now aim to study the efficacy of G-CSF mobilized granulocytes in patients.

SP806

A systematical dose – response analysis on lenograstim plus dexamethasone for neutrophil mobilization and collection for transfusion

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OBJECTIVE: To evaluate the dose – response relationship of Lenograstim plus dexamethasone for neutrophil mobilization and collection for transfusion. **STUDY DESIGN:** In a prospective study, 260 healthy volunteers received oral dexamethasone (8 mg) plus a single subcutaneous injection of glycosylated G-CSF (Lenograstim) at medians of 1.5 (1.0-2.3) μg/kg (n=43),

3.1 (2.4-4.1) µg/kg (n=73), 6.0 (4.3-7.9) µg/kg (n=123), and 12.0 (8.2-17.2) µg/kg (n=21), and underwent neutrophil aphereses using the Spectra PMN program. We compared WBC and PMN mobilization and collection results and evaluated the severity and clinical significance of donor adverse reactions. Fifty-two neutropenic patients (29 children, 23 adults) underwent 271 neutrophil transfusions (GTX) every other day to maintain peripheral WBC levels constantly above 500/µL. **RESULTS:** Within the dose range 1.5 / 3 / 6 µg/kg each doubling step was associated with a 10-15% PMN increase in peripheral blood up to 32.8 (19.1-49.2) $\times 10^9$/L (6µg/kg; p≤0.00032) as well as in the neutrophil concentrate up to 79 (34-150) $\times 10^9$/U (6µg/kg; p≤0.00042). A further doubling to 12µg/kg achieved neither better mobilization nor better apheresis results. The rate of clinically important adverse reactions increased already with the 6µg/kg mobilization step. The GTX resulted in peak WBC increments to 3.8 (0.4-18.2) $\times 10^9$/L (children) and 1.6 (0.3-9.4) $\times 10^9$/L (adults), but in adults the WBC threshold of 500/µL was not continuously overcome. **Conclusions:** The most effective dose / response ratio for PMN mobilization was demonstrated in the 6 µg/kg Lenograstim group. In neutropenic adults GTX treatment on an every other day schedule may be ineffective.

SP9: Immunohematology II – Granulocytes/HLA

SP901

Transfusion-Related Acute Lung Injury (TRALI)

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During the last decade, increasing numbers of TRALI cases have been reported by the haemovigilance systems of many countries. According to recent SHOT and FDA data, TRALI has become the second / third most common cause of transfusion-associated death and major morbidity in the UK and the USA, respectively. In a series of 36 patients, 72 percent required mechanical ventilation. Six to 13 percent of TRALI cases are fatal. Since there were discrepancies in the classification of pulmonary transfusion reactions as TRALI, a working party of the European Haemovigilance Network was established to develop a common definition of TRALI based primarily on clinical symptoms and radiologic appearance. This definition includes respiratory distress, bilateral lung infiltrations in chest radiograph during or within 6 hours of the completion of the transfusion and no indication of transfusion-associated circulatory overloads (TACO), transfusion-associated dyspnea (TAD), or allergic dyspnea. Further symptoms frequently observed in TRALI were hypoxemia, fever, tachycardia and hypotension. Concerning pathogenesis, two subtypes are distinguished: antibody-mediated (immunogenic) and non-immune TRALI. About 80 percent of TRALI cases are caused by leucocyte antibodies in donor blood directed against HLA and HNA (Human Neutrophil Allo-) antigens. Occasionally, in TRALI cases after transfusion of non- leucocyte-depleted red blood cells, the leucocyte antibodies were found in the recipient`s blood. Implicated donors were usually multiparous women. Immunogenic TRALI mainly occurred after transfusion of fresh frozen plasma whereas non-immune TRALI developed after transfusion of cellular blood products. To prevent further TRALI reactions, fresh frozen plasma and platelet concentrates from alloimmunized donors involved in TRALI cases should not be transfused any more.

SP902

Genetic Associations of Primary Autoimmune Neutropenia

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Purpose: The aetiology of primary autoimmune neutropenia not known. In contrast to acute ITP in childhood, a trigger such as an acute viral infection before onset of ITP could not be identified. However, the frequent formation of HNA-1a specific autoantibodies suggests a genetic predisposition. In fact, autoimmunization against the neutrophil specific antigen HNA-1a (NA1) was reported to be associated with HLA-DR2. However, HLA class II typing was performed serologically and only a low number (N=26) of affected infants was tested. In addition, this work was restricted to HNA-1a specific autoimmunization. Therefore a more extensive patient group was investigated for candidate genes influencing immune response. **Methods:** We analysed 105 neutropenic infants (< 3 years) with clinically and serologically confirmed primary autoimmune neutropenia irrespective of autoantibody specificity. We typed them the FCGR3B (HNA-1), FCGR2A (Fc gamma receptor IIa, H131/R131) and HLA class II alleles using PCR-SSP methods. Statistic analysis was done by chi-square test with Bonferroni correction for multiple tests. **Results:** As expected, the FCGR3B*01 (HNA-1a, NA1) allele was highly significantly associated with primary autoimmune neutropenia (p<0,001). Concerning the FCGR2A alleles, the H131 allotype was significantly associated (p<0,05). Unexpectedly, we found only a significant negative association with HLA DRB1*04 alleles primary autoimmune neutropenia (p<0,05). DRB1*01 alleles were markedly but not significantly more frequent. The frequency of DRB1*14 alleles was increased threefold compared to the controls. **Conclusion:** Our results confirm that the HNA-1a polymorphism of the Fc gamma receptor IIIb predisposes for autoantibody formation. This seems to be facilitated if the protective HLA DRB1*04 alleles are absent.

SP903

Variable expression of NB1: genetic variants and correlation between surface and total NB1 expression

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The human neutrophil NB1 glycoprotein (CD177, HNA-2a) has gained clinical importance as a target in alloimmune neutropenias and in TRALI. In addition, NB1 is a hematopoietic cell surface receptor and overexpressed in patients with polycythemia rubra vera (PRV). NB1 can be found on plasma membranes of neutrophils as wells as in secondary granules. The expression of NB1 on the cell surface is heterogeneous, and the size of the NB1-negative subpopulation varies between individuals. Variability in the total NB1 protein content of granulocytes has not been investigated. In this study, we sought to explore *(1)* whether there is a correlation between NB1 surface expression and total protein content and *(2)* whether different alleles of the NB1 gene are associated with the heterogeneous expression of NB1. The size of the neutrophil subpopulation of 100 donors as well as the number of NB1 molecules per granulocyte was measured by flow cytometry. An ELISA system was established to measure the total NB1 protein content in granulocyte lysates. Dilutions of soluble NB1 derived from CHO transfectants were used as standards. The NB1 gene from 16 donors was sequenced, and DNA from all donors was subsequently typed by PCR-SSP for selected polymorphic positions. The relevance of inter-individual differences in the correlation between NB1 surface expression and total protein content on one hand and the likelihood of genetic regulation on the other hand will be discussed.

SP904

Apoptosis as a mechanism in neonatal alloimmune neutropenia

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The mechanisms by which neutrophils are removed from the circulation in patients with neonatal alloimmune neutropenia (NAIN) have not been elucidated. Alloimmunization against human neutrophil antigen (HNA-)4a leads to the production of anti-Mart antibodies. However, NAIN is not observed in all children affected. Here, we present evidence that antibodies against HNA-4a are heterogeneous and can differ between individuals although a single amino-acid exchange controls the expression of HNA-4a epitopes. Two types of anti-Mart antibodies could be defined: Type 1 Mart alloantibodies are able to inhibit the formation of platelet-neutrophil aggregates and to impair the adhesive properties of neutrophils. In addition, type 1 Mart alloantibodies can efficiently prime neutrophils for the production of reactive oxygen species (ROS), a final common mediator of apoptosis. In contrast, type 2 Mart alloantibodies do neither interfere with neutrophil adhesiveness

nor do they influence ROS production. Two general conclusions can be drawn from these findings: *(a)* all polymorphism-"specific" human alloantibodies are not alike, but can differ in their functional properties; (b) sustained ROS generation and subsequent apoptosis of neutrophils is a hitherto overlooked, but possibly clinically important mechanism of cell removal in antibody-mediated cytopenia.

SP905

Stable knock down of non-acceptable HLA mismatches in solid organ transplantation

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Purpose: HLA polymorphism represents the most relevant immunologic barrier in organ and stem cell transplantation. As RNA interference (RNAi) is capable of effectively knocking down specific transcripts, RNAi was used to selectively reduce cellular HLA class I expression. Methods: Small interfering RNAs (siRNAs) were designed and chemically synthesised to target both β2-microglobulin (β2m) as well as the heavy chain transcripts. Sensitive sites in the target RNAs were identified using an in vitro translation system. The best siRNAs were used for knockdowns in B-LCL, K562 and HeLa cells. In order to achieve a stable reduction of HLA expression, lentiviral expression vectors were constructed encoding the sequences for short hairpin RNAs (shRNAs), which are subsequently processed intracellularly into functional siRNAs. Expression of HLA and β2m was determined by flow cytometry. Results: Transfection of B-LCLs and K562 cells with siRNA targeting β2m resulted in a 50% suppression of β2m after 5 days. Using group- and gene-specific siRNAs targeting HLA-A transcripts, a reduction of up to 70% was observed after 6 days when performing repetitive transfections. When lentiviral vectors were used for transduction of shRNA, HLA-A suppressions of 70% in HeLa cells and 60% in B-LCLs were obtained, after 6 days without observing off-target effects. Conclusions: The present data strongly support the idea that knocking down HLA expression in class, gene and group-specific ways is extremely effective. The possibility to deliver the constructs by viral transduction, which offers both a stable transcript reduction and organ selective applicability, presents an exciting new immunotherapeutic approach in the field of organ and stem cell transplantation.

SP906

Definition of allele-specific peptide motifs to estimate the severity of HLA mismatches

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Purpose: In this study we have sequenced peptides eluted from truncated recombinant HLA-A*6602 and A*6603 molecules. **Methods:** Peptides were eluted from truncated recombinant HLA-A*6602 and A*6603 molecules and their features were compared with data reported for peptides presented by A*6601. **Results:** The A*6601 allele differs from A*6602 by two amino acid (AA) exchanges at positions 90 (outer loop) and 163 (pocket A). A*6603 differs from both alleles by an additional exchange at position 70 (pockets A and B). There were no significant differences in the peptide motifs of A*6602 and A*6603, suggesting a minor importance for the exchange Gln70→His, with both AA having neutral side-chains. However, we observed a striking difference at the auxiliary anchor P1 of peptides bound by A*6601 (polar/acidic AA: Asp, Glu) and A*6602/6603 (polar/ neutral AA: Ser) which interacts with pocket A. This finding may be best explained by the exchange Arg163→Glu which results in a shift towards higher acidity in pocket A of A*6602/6603, apparently leading to the loss of preference for acidic auxiliary anchors. **Conclusions:** The similar peptide motifs of A*6602 and A*6603 suggest low allogenicity when these alleles are mismatched in stem cell transplantation; whereas differences in the peptide motif suggest the opposite for the mismatch A*6601 vs. A*6602/6603. This knowledge about allele-specific peptide motifs will contribute to the ranking of HLA subtype mismatches in the donor selection process, when no HLA-identical donor is available.

FS1: Anniversary Symposium: 10 Years ARGE-KMSB

FS102

Die Aufgabe der Blutspendedienste in der regionalen Betreuung von Knochenmarkspenderwilligen.

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Im Rahmen der wissenschaftlichen Tagungen der Sektion Transplantation der DGTI wurden bereits Anfang der neunziger Jahre die Rolle und Aufgaben der Blutspendedienste (BSD) bei der Rekrutierung freiwilliger nichtverwandter Knochenmarkspender erörtert. Folgende Aspekte wurden diskutiert und führten am 08.10.1992 während des DGTI-Symposium in Berlin zur Gründung der ARGE-KMSB.
Blutspender gelten als sozial hochmotivierte und zuverlässige Personen. Ihre Bereitschaft zur Knochenmarkspende ist nachweislich sehr hoch. Die meisten Blutspender kommen mehrmals jährlich zum Blutspenden. Somit ist ein kontinuierlicher Kontakt mit dem BSD vorhanden, sodass eine regelmäßige medizinische und infektionsrelevante Überwachung zur Eignung als Blut- aber auch Knochenmarkspender gewährleistet wird.
Die BSD erfassen 3–6% der deutschen Bevölkerung und haben Erfahrung bei Aufklärung, Rekrutierung und Motivierung von Spendern. Über 50 Institute in Deutschland garantieren eine flächendeckende, hausnahe Spenderbetreuung.
- Labormethoden der Spenderauswahl sind etabliert. (Histokompatibilitätstestung, infektionsrelevante Untersuchungen, Blutgruppenbestimmungen, etc.)
- Kenntnis der Materie aus der täglichen Arbeit (Substitutionstherapie in der Hämatologie, Onkologie, KMT, etc.)
- Praktische Erfahrung bei der patientenbezogenen Knochenmarkspenderauswahl
- Bereits vorhandene Knochenmarkspender-Dateien in über 30 BSD.

Angaben zur Spenderentwicklung und zur Anzahl der wirklich geleisteten Blutstammzell-Spenden werden aufgelistet und in Beziehung zum gesamten Knochenmarkspenderwesen in Deutschland gesetzt. Inwieweit die Blutspendedienste die vorhandene Fachkompetenz wahrnehmen und die oben beschriebenen Aufgaben seit 1992 erfüllen wird dargestellt und diskutiert.

FS103

The typing, search and evaluation of unrelated stem cell donors – a challenge for institutions in Transfusion Medicine to assure allogeneic stem cell transplantations

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Nowadays, HLA-compatible unrelated stem cell donors (URD) can be identified in nearly 80% of the cases for recipients undergoring allogeneic stem cell transplantation (SCT). All German Donor Registries and Search Centers are connected with the ZKRD (Zentrales Knochenmarkspender-Register) in Ulm in order to achieve a fast worldwide mediation between compatible donors and needy recipients of stem cells.
Donor Registries
Many Departments of Transfusion Medicine are involved in the information, registration and evaluation of potential stem cell donors within German Donor Registries. Just like for blood donors, the acquisition of suitable donors including the medical check and the exclusion of donors with risk factors represent an important and responsible task. The percentage of compatible donors within single registries can distinctly be enhanced by skilful HLA-typing strategies, e. g. by high resolution HLA-class II typing or additional HLA-Cw typing.
Donor search centers
Donor Search Centers are associated with the Transplantation Units. In nearly 50% of the German Donor Search Centers, Departments of Transfusion Medicine take part in logistic or diagnostic affairs for evaluation of stem cell donors. Optimal search strategies not only comprise the HLA-compatibility and the exclusion of relevant infectious donor diseases but also consider further compatibility criteria (gender, CMV status, etc.) and economic necessities of donor searches.

Collection Centers
These days, peripheral blood stem cells (PBSCs) are primarily used for allogeneic SCT. The apheresis of PBSCs takes place at Collection Centers belonging either to a Donor Registry or performing the aphereses by order of a Donor Registry. Many Departments of Transfusion Medicine have long standing experiences with the collection and quality control of PBSCs. In certain cases, the manipulation of PBSC products, like the depletion of T-lymphocytes, becomes necessary. Again, the assessment of donor risks and the medical care and aftercare are tasks for specialists of Transfusion Medicine working in this field. Finally, the release of donor and transplant must be organized in a professional manner.
ZKRD-Standards
In order to assure the conditions for the selection of best compatible donors and for a fast provision of recipients with suitable stem cell transplants, new "ZKRD-standards" for the accreditation of participating centers are currently in preparation. The experiences from Departments of Transfusion Medicine to improve the safety and quality of "classical blood components" may also contribute to ameliorate the handling with allogeneic stem cell transplants.

Thursday, September 23, 2004: Poster Sessions

PS5: Autologous and Blood-Saving Procedures

PS501

Safety and Efficacy of Unmodified Whole Blood Versus Buffy Coat-Depleted Red Cell Concentrates in Autologous Transfusion of Elective Orthopaedic Surgery Patients

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Purpose: Storing autologous blood as whole blood has been proposed for increasing the cost-effectiveness of preoperative autologous blood donation programmes. However, experimental data suggest that autologous leukocytes might lead to immunomodulation similar to the effect attributed to allogeneic leukocytes. The results of published clinical studies are unreliable due to small patient numbers. **Methods:** In a retrospective analysis, the postoperative outcome of 120 patients undergoing elective orthopaedic surgery and having donated up to two units of autologous whole blood (AWB group) was compared with a control group of 52 patients whose autologous donation had been processed into buffy coat-depleted red cell concentrates (ARCC group). At least one autologous unit, but no allogeneic units, had been transfused in all analyzed patients. **Results:** No significant differences in baseline characteristics (age, gender, body mass index, ASA score, spectrum of performed operations) were found between the groups. Donation schemes were equally efficacious in both groups (median preoperative haemoglobin 125 g/L in both groups, P = 0.90). There was no significant difference in postoperative infection rates between the two groups. Overall rates were 8.3 % in the AWB group and 7.7 % in the ARCC resulting in an age-adjusted odds ratio (OR) of 1.08 (P = 0.89). Surgical (OR 0.79, P = 0.55), thromboembolic (OR 0.91, P = 0.89) and other recorded complications, length of postoperative hospital stay (P = 0.98) and days of use of antibiotics (P = 0.21) were also not significantly different between the groups. If patients additionally receiving allogeneic blood were included in the analysis surgical experience was found as main predictor of the risk for allogeneic transfusions (junior vs. senior consultant OR 5.26, P = 0.003). The type of autologous blood product provided was not associated with an increased risk for allogeneic transfusions. **Conclusions:** The results of this study suggest that transfusion of up to two units of unmodified autologous whole blood is as efficacious as the transfusion of buffy coat-depleted autologous red cell concentrates and does not negatively influence the postoperative outcome in elective orthopaedic surgery.

PS502

The Influence of Autologous Leukocytes on Postoperative Immune Function after Transfusion of Non-Leukocyte-Depleted Autologous Whole Blood

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Purpose: Experimental data suggest that leukocytes (WBC), WBC and platelet degradation products, and soluble factors (e. g. HLA class I and Fas ligand molecules) accumulating in autologous whole blood (AWB) during storage might lead to transfusion-related immunomodulation (TRIM). Results of clinical studies are lacking. **Methods:** Patients undergoing preoperative autologous blood donation (2 x 500 ml units 3-5 weeks prior to surgery) and scheduled for elective hip replacement surgery were randomly allocated to receive leukocyte-depleted (LD) or non-LD AWB. WBC and lymphocyte subsets were determined for transfused patients, using flow cytometry, prior to first donation (baseline), prior to surgery, and on day 1, 5 and 10 after surgery. **Results:** In the whole group (N=58), i. e. transfused and not transfused patients, considerable changes of WBC subsets occurred postoperatively: granulocytes and monocytes increased by 53 % and 38 %, respectively; lymphocytes decreased by 30 %. The B-cell portion of the lymphocytes increased from 13.1 % to 17.6 %, P < 0.0001; all other lymphocyte subsets slightly decreased. These changes occurred shortly after surgery and had not returned to baseline even after 10 days post surgery. No significant differences between patients receiving non-LD AWB (n=10) vs. LD AWB (n=15) could be found for granulocytes, lymphocytes and lymphocyte subsets (T-, B-, NK-, CD4+-, CD8+-cells, CD4/CD8-ratio). Mean postoperative monocyte counts were significantly higher in the non-LD group (8.8 % vs. 7.1 %, P < 0.05). **Conclusions:** The preliminary results of this study suggest that transfusion of non-LD AWB leads to an additional and lasting increase in monocyte counts exceeding the immunomodulatory effect attributable to surgery alone. Further studies are necessary to elucidate whether these changes result in a clinically relevant TRIM effect and whether this effect is supposed to protect against or to promote postoperative infectious complications.

PS503

Leukodepleted Whole Blood: Suitable for Homologous Use?

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BACKGROUND: Filtered whole blood (FWB) is already widely used for autologous transfusion. If quality of whole blood could be improved to component-like requirements by leukodepletion, FWB could become an interesting option for homologous use, too, mainly massive transfusion. **DESIGN:** Of the 32 units of whole blood donated by healthy volunteers according to German guidelines 16 were filtered prestorage and stored for up to 49 days. The 16 unfiltered units and 14 units of filtered red blood cell concentrates (FRBC) served as controls. Several in vitro parameters indicative of product quality were determined at designated times. **RESULTS:** Apart from significant differences in haematocrit (56.2±3.6 vs. 37.9±3.9%), and plasma concentrations of free haemoglobin (93.1±37.8 vs. 57.8±24.3 g/dL), K+ (38.9±5.3 vs. 31.5±4.3 mmol/L), and ATP (2.7±0.2 vs. 1.6±0.4 µmol/g Hb) with higher levels in FRBC, no remarkable differences were observed with haemolysis (0.23±0.07% vs. 0.31±0.13) and pH value (6.63±0.03 vs. 6.62±0.02) between FRBC and FWB at storage end. Lack of leukodepletion manifested in significantly higher rates of haemolysis (0.44±0.21%), free haemoglobin (89.6±43.5 g/dL), and lower pH values (6.56±0.04). During 42 days of storage, sufficient amounts (% of the initial mean value) were observed with stable (F XI 97.5%) and labile clotting factors (F V 92.9%, F VIII 69.2%), and inhibitors (AT III 89.0%) without any signs of activated coagulation. **CONCLUSION:** Our data indicate a quality of FWB comparable to components during 42 days of storage. Thus, FWB could be an interesting strategy to facilitate and economize blood supply, especially for surgical or trauma patients. This, however, remains to be investigated by means of clinical studies.

PS504

The effect of Aspirin ingestion by blood donors on in vitro properties of stored platelet concentrates

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Purpose: Preventing the activation of platelets may ameliorate (or mitigate) the platelet storage lesion (PSL), which encloses all structural and biochemical changes caused by collection, processing and storage of platelet concentrates (PC). Partial inhibition of platelet function due to ingestion of Aspirin (ASA) by blood donors reduces the functional activity of the collected platelets, however by preventing premature platelet activation it might as well reduce the PSL. We therefore investigated potential interactions of ASA ingestion by blood donors on platelet activation during apheresis and in vitro properties of stored platelet concentrates. **Methods:** In a randomized cross-over study, 10 healthy donors donated 2 apheresis platelet concentrates (APC) each, taking 500 mg ASA 12 hrs prior to one of the aphereses (group A), and taking no medication prior to the other donation (group B). In vitro tests of platelet function were performed in donors before and after apheresis and in APCs during storage (days 1, 3 and 5). **Results:** ASA ingestion resulted in a significant decrease of induced platelet aggregation in donors ($p<0.005$) and APCs on day 1 ($p<0.01$). TRAP-6 induced expression of P-Selectin (CD62p) was significantly reduced in group A APCs only on day 1 ($p<0.02$). There were no significant differences of in vitro function (LDH, lactate, pH, morphology score, CD62p expression, fibrinogen binding) between group A and B (APCs and donors). Apheresis did not result in a significant activation of platelets in donors or APCs. **Conclusions:** These limited data do not show a detectable beneficial effect of ASA ingestion on the PSL but do suggest that ASA ingestion pre-apheresis may not be detrimental to the clinical effectiveness of the stored product.

PS505

Effectiveness of preoperative autologous blood donation (PABD): the effect of donation schedule on hemoglobin regeneration and hemoglobin recovery

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Purpose: PABD is an accepted procedure to reduce the need for allogenic transfusion and the associated risks. Frequent PABD's and a short interval between last donation and surgery leads to anemia. The aim of this study was to test the effects of different donation schedules on hemoglobin regeneration and hemoglobin recovery after PABD of 2 and 3 units. **Methods:** In a retrospective analysis we have studied 427 patients who donated autologous blood before elective orthopedic surgery. Patients were divided into 5 groups depending on different donation intervals and the number of donated units (table). The regenerated hemoglobin mass (Hb_{reg}) was calculated as follows:
$Hb_{reg} = Hb_{preop} \times GBV - (Hb_{init} \times GBV - Hb_{init} \times 0.48l - Hb_2 \times 0.48l - Hb_3 \times 0.48l)$

Results:

parameter	group 1	group 2	group 3	group 4	group 5
n-m/f	123-48/75	77-39/38	84-28/56	73-48/25	70-48/22
donated units (480ml/ unit)	2	2	2	3	3
intervall donation 1-2 (days)	7.6	3.6	3.0	8.2	3.5
intervall donation 2-3 (days)	-	-	-	6.9	6.6
interval between last donation and surgery (days)	7.8	15.3	21.9	8.2	15.4
regenerated hemoglobin mass (%)	46.9	64.7	97.5	52.6	74.0

Only patients of group 3 showed nearly complete regeneration of donated hemoglobin mass (97.5%). Patients of group 5 regenerated more hemoglobin (74.0%) after PABD of 3 units than patients of group 4. In this Group the difference between donated hemoglobin mass and regenerated mass was 48.5g. At the 3. donation an average amount of 57.9g was drawn from the patient. This means (in a mathematical sense) only 16.2% of the 3. unit was regenerated. **Conclusion:** Hemoglobin regeneration after PABD with a conventional schedule (group 1 and 4) is ineffective. A short interval (3-4 days) between two donations, which induces a rapid fall of the hemoglobin ratio, leads to a stronger and faster stimulation of erythropoiesis. The intervall between last PABD and surgery should be maximized. Thus, a nearly complete hemoglobin recovery can be expected after PABD of 2 units with an interval of 3 weeks between last donation and surgery. We suggest that more than 2 units cannot be regenerated without additional interventions in the given time course.

PS506

Preoperative Autologous Blood Donation in Germany: Is it Worth the Costs? – Is it Worth the Risks?

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Purpose: Preoperative Autologous Blood Donation (PABD) has been widely established and used in Germany following the 1991 decision of the Federal Supreme Court (BGH). However, transfusion safety has been dramatically improved since then. HIV and HCV infections by allogeneic blood transfusion, which were a major threat in the 80ths, are almost negligible risks nowadays (residual risk for HIV = 1:20 million; for HCV: less than 1:20 million). **Methods:** We compared infectious risk markers for HBV, HIV and HCV as well as bacterial contaminations in autologous versus homologous donations in our Frankfurt donor population from 1996/1997 to 2002/2003. Testing for viral infections were performed using serological as well as PCR techniques. Bacterial contamination was tested by incubation. **Results:** Out of 19,851 autologous samples tested, 0.232% were tested positive for HBV, 0.343% were tested positive for HCV and 0.005% were tested positive for HIV. In comparison, out of 1,307,170 homologous samples tested, 0.02% were tested positive for HBV, 0.02% were tested positive for HCV and 0.001% were tested positive for HIV. Bacterial contaminations were detected in 0.32% of the autologous samples compared to 0.06% of the homologous samples (313 autologous samples vs. 4,966 homologous samples tested 1997-2003). Analysis of a greater number of samples led to comparable results. **Conclusions:** Autologous blood products are of lower quality compared to homologous blood products regarding infectious risks. Reasons for these findings comprise the following: a.) Autologous blood donors are older patients with multiple diseases, drug intake and procedures performed; b.) A major proportion of autologous blood donors are first time donors compared to >90% multiple donors in our homologous blood donor population; c.) Autologous blood products are older compared to homologous products due to the underlying principle. Therefore, autologous donations should nowadays be restricted to patients with complex immuno-hematological problems. Regulations and laws should be amended or modified accordingly. Autologous donations with positive infection markers must not be stored, but should be destroyed.

PS507

Management of peri-operative blood supply for cancer surgery in a case of anti-Cellano, anti-C and anti-D; forced pre-operative autologous blood donation (PABD) and search for compatible blood

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A 77-year-old man in good general condition with a second relapse of cancer of the rectum was scheduled for curative proctectomy following previous radio-chemotherapy with 5 FU and 50 Gy. The patients blood group was O-Rh negative (ccddEe). Pre-operative antibody screening for irregular erythrocyte antibodies was positive. Anti-Cellano, anti-D and anti-C were identified and an a further antibody was suspected. The peri-operative demand for transfusion was estimated at 4 RBC (not regarding bleeding complications). We decided to perform forced PABD and simultaneously started an extensive search for suitable blood donors. We mailed an inquiry for O-Rh neg KK RBCs to 152 transfusion services (TS) in Germany, Austria and Switzer-

land (135 D, 8 A; 9 CH). 68 TS responded, 36 gave negative reports, 4 TS had suitable RBC in stock (n=7), 24 TS had suitable donors (n=56) and 3 TS had deep frozen RBCs (n=?). We received 15 blood samples which all were crossmatch negative, so that an additional antibody could be ruled out. Finally, we ordered 5 RBCs to be kept in reserve for bleeding complications. PABD was started six weeks before the scheduled operation. Recombinant human erythropoietin (rHuEPO) was administered s.c. at a dose of 3 x 125 IU/kg thrice weekly combined with i.v. supplementation of 200 mg ferrous gluconate twice weekly. Starting from an initial HB of 119 g/l, five units of RBC could be collected at weekly intervals. The HB never dropped below 111 g/l and the preoperative HB was 131 g/l. The patient received 4 units of autologous RBC (2 during surgery and 2 the following day) and left the hospital three weeks after operation (HB 118 g/l). Forced PABD using rHuEPO and intravenous iron supplementation was effective in providing high yields of autologous blood even in a cancer patient after radio-chemotherapy. However, with respect to the considerable costs, this therapy should be performed only in selected cases. Looking for suitable donors was successful, but very time-consuming. A nation wide registry for rare donors would be desirable.

PS508

Perioperative blood salvage is more efficacious than preoperative autologous blood donation

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Purpose: Peri-operative blood salvage (PBS) and preoperative autologous blood donation (PABD) are established blood conservation measures. While PABD can be applied in elective surgery, exclusively, and is associated with a high discarding rate, PBS can be applied both in elective and emergency cases, however, is considered an expensive measure. No data have been published so far comparing/calculating efficacy of either measure. Methods: Prospective analysis of data from 693 orthopedic pat. with PABD concerning increase in total RBC-mass (+RBC). After calculating each individual pat.'s +RBC due to PABD, individual blood-loss (BL) that could be covered by re-transfusion of +RBC was calculated by applying the following mathematical re-transfusion concept that is described elsewhere (1): Re-transfusion of +RBC to maintain hct min at the desired level (either 24, 21, or 18 percent) during ongoing BL, and securing normovolemia by additional infusion of colloid. These data were compared with a PBS-model with an assumed recovery of 30 percent of RBC lost intraoperatively (PBS30) until reaching hct min. The same mathematical re-transfusion concept was applied as with PABD. Statistically significant differences (*) between PABD and PBS (by t-/U-test according to Gaussian distribution) were adopted when $p<0.05$
Results: Tab. 1 summarizes relevant data.

hct min	18%	21%	24%
n: m/f (%)	693: 282/412 (40.5%/59.5%)		
age (yrs)	62.7 ± 10.8		
pat. blood vol. (L)	4.6 ± 0.8		
pat. RBC mass (L)	1.8 ± 0.4		
hct init (%)	39.2 ± 3.3		
hct preop. (%)	36.9 ± 2.7		
+RBC(PABD)(ml)	164 ± 11*		
+RBC (PBS) (ml)	431 ± 134*	346 ± 116*	273 ± 100*
δ +RBC (ml)	267 ± 178	183 ± 166	109 ± 156
BL 'PABD' (L)	4.7 ± 1.1*	3.8 ± 0.9*	3.1 ± 0.8
BL 'PBS' (L)	6.0 ± 1.6*	4.5 ± 1.3*	3.4 ± 1.1
δBL (L)	1.2 ± 0.8	0.7 ± 0.6	0.3 ± 0.5
PABD superior to PBS - n (%)	23 (3.3%)	83 (12%)	198 (29%)

Conclusions: Mathematical comparison of PABD and PBS demonstrates PBS superior to PABD concerning +RBC, and substituting for blood-loss at different hct min-levels. The lower hct min, the more efficacious is PBS, and the lower the percentage of patients taking advantage from PABD.

Reference: 1. Singbartl K et al.: J Surg Res 1999; 86: 206 – 212. *Erratum* in J Surg Res 2000; 88: 215.

PS509

No evidence for increased intraoperative bleeding in aortic-valve stenosis: a comparative analysis of hemotherapy in 136 patients undergoing aortic-valve replacement

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Purpose: An association of aortic-valve stenosis and abnormal bleeding due to acquired von Willebrand disease has been reported. In this setting, high-shear stress generated by the transvalvular gradient leads to a conformational change of plasmic von Willebrand factor, making the adhesive protein more susceptible for proteolytic cleavage. Considering the high prevalence of this acquired hemostatic defect (approximately 80%) in patients with severe aortic-valve stenosis, affected patients should also have increased intraoperative bleeding reflected by the number of intraoperatively transfused blood components. **Methods:** To assess the role of aortic-valve stenosis as a risk factor for intraoperative bleeding, we compared the mean number of intraoperatively transfused red-blood cell units, fresh-frozen plasma units, and platelet concentrates during valve replacement for aortic-valve stenosis (n=50), aortic-valve insufficiency (n=19) and combined aortic-valve defects (n=67). **Results:** The three subgroups did not differ significantly regarding the mean number of transfused red-blood cell units (0.94 ± 1.36, 0.4 ± 0.9, or 0.86 ± 1.3, respectively) and plasma units (0.04 ± 0.28, 0.21 ± 0.71, or 0.15 ± 0.61, respectively). None of the patients received platelet concentrates. A multivariate logistic regression model adjusted for age and gender did not show an influence of the presence and severity of aortic-valve stenosis on intraoperatively applied hemotherapy. **Conclusions:** Our data demonstrate that aortic-valve stenosis is not a major risk determinant for intraoperative bleeding as assessed by intraoperative hemotherapy. Thus, the findings question the role of aortic-valve stenosis as a risk factor for intraoperative bleeding. In addition, considering the high rate of acquired von Willebrand disease reported in this setting, our results raise the question of the clinical relevance of shear-stress induced von Willebrand factor abnormalities.

PS6: Production of Blood Components and Quality Assurance

PS601

Exposure of apheresis donors to di(2-ethylhexyl)phthalate (DEHP)

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Purpose: Most disposables used for apheresis techniques are manufactured from polyvinylchloride (PVC). To make the PVC polymer flexible the plasticizer di(2-ethylhexyl)phthalate (DEHP) is added. DEHP leaches out into the blood contacting the PVC surface. That way the donor is exposed to DEHP. DEHP is a known reproductive and developmental toxicant in animals which effects the development of the male reproductive system. DEHP might also disrupt endocrine regulation of the reproductive system in humans. **Methods:** We determined the exposure of apheresis donors to DEHP. We collected the urine of 6 plasmapheresis donors, 6 single-needle discontinuous-flow plateletpheresis (DFP) donors, 6 dual-needle continuous-flow plateletpheresis (CFP) donors and 5 controls pre and during 24 hours after the procedure. We determined three specific urinary DEHP metabolites 5OH-MEHP: mono(2-ethyl-5-hydroxyhexyl)phthalate; 5oxo-MEHP: mono(2-ethyl-5-oxo-hexyl)phthalate and MEHP: mono(2-ethylhexyl)phthalate. **Results:** We found significantly ($p<0.0001$) elevated urinary levels of all three DEHP metabolites for DFP- and for CFP-donors compared to plasmapheresis donors and controls. Highest metabolite concentrations were observed in the urine samples taken shortly after the CFP procedure and were in the mg/L range (5OH-MEHP: 1346 µg/L; 5oxo-MEHP: 1153 µg/L; MEHP: 536 µg/L). DEHP exposure for CFP was significantly higher than for DFP ($p<0.0001$). Mean DEHP doses were calculated to be 1.18 mg for DFP- and 2.1 mg for CFP-donors. DEHP exposure for plasmapheresis

(0.37mg) was in the range of the controls (0.41 mg). All calculated DEHP intakes caused by CFP (28.2-38.1 µg/kg body-weight/day) exceeded the reference dose (RfD) of the U.S. Environmental Protection Agency (EPA) of 20 µg/kg body-weight/day. One volunteer exceeded the tolerable daily intake (TDI) of 37 µg/kg/day of the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) of the European Commission .**Conclusion:** DEHP exposure due to apheresis considerably adds up to the general and omnipresent DEHP exposure. This can lead to a margin of safety (MOS) much smaller than 100 for some individuals. At present, to avert conceivable health risks from plateletpheresis donors, discontinuous-flow devices should be preferred. Strategies to avoid DEHP exposure of donors during apheresis must be developed.

PS602

Adding platelet additive solution to highly concentrated platelets at different times – is there an impact on invitro quality of platelets?

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Purpose: Apheresis PLT concentrates in PLT additive solutions (PAS-APCs) have become the focus of interest. With increasing use of PAS-APCs questions arise on possible impact of manipulations on PLT quality. We compared in vitro parameters of PAS-APCs with different hold times prior to addition of two PAS. **Methods:** 20 blood donors underwent two PLT apheresis with Trima ACCEL® (interval >/= 2 weeks) collecting an APC (4000x103 PLT/µl in 150 ml plasma) at a time which was split into 2 single products. Either 140 ml of PAS II (donation 1) or PAS IIIM (donation 2) was added after 2 or 8 h hold time resulting in PAS-APCs of 225 ml concentrated at 1400x103 PLT/µl and PAS proportion of 35%. Lactate, glucose, pH, LDH, CD62-expression (CD62), and hypotonic shock response (HSR) were tested on day 1, 5 and 7. **Results:** Data are given in table 1. **Conclusions:** PLTs stored in PAS IIIM maintain better function and cell integrity than PLTs in PAS II. Although difference in hold time of 6 h does not have an impact on PLT function there seems to be an influence on PLT metabolism as PLT with a 2 h hold time showed higher lactate production and glucose utilisation.

TABLE 1. In vitro quality of stored PLTs

	2h PAS II meanSD	8h PAS II meanSD	P-value	2h PAS IIIM MeanSD	8h PAS IIIM meanSD	P-value
HSR day 5 (%)	50.221.9	55.320.8	0.297*	58.619.8	55.918.0	0.592*
HSR day 7 (%)	44.219.8	51.221.7	0.304*	62.120.8	67.932.6	0.463*
CD62 day 5 (%)	34.79.3	35.28.9	0.707*	10.74.8	11.16.8	0.780*
CD62 day 7 (%)	37.45.8	39.56.5	0.159*	16.75.9	17.16.8	0.725*
pH at 22°C day 5	7.240.09	7.180.12	0.043	7.470.06	7.380.13	0.002
pH at 22°C day 7	7.280.17	7.230.17	0.004	7.470.08	7.400.12	0.004
LDH day 7 (U/l)	21269.9	21583.4	0.833*	14538.8	13844.7	0.350*
Glucose utilization per day per 10^6 PLT (mg)	12.22.4	11.22.8	<0.001	10.13.9	9.184.0	0.001
Lactate increase per day per 10^6 PLT (mg)	6.21.3	5.81.6	<0.001	4.42.0	4.02.5	<0.001

* not significant

PS603

Double-dose-RBCs collected with ALYX: A paired Comparison to Standard RBCs

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Purpose: Blood component collection by apheresis is considered as means to optimise donor pool utilisation, product standardisation and quality. In this study the performance of the new mobile apheresis device ALYX with respect to RBC quality and variability is compared to conventionally prepared RBCs in a strictly paired fashion. **Methods:** 32 male blood donors were enrolled for two subsequent blood donations: One of which conventional RBC collected in CPD/SAG-M were prepared and 10 weeks later a double-dose RBC donation collected with the component collection system ALYX that utilises ACD/SAG-M. One bag of each ALYX donation was irradiated. A paired comparison for total hemoglobin content, hematocrit, hemolysis, pH, ATP, 2,3-DPG, glucose, lactate, potassium, total supernatant protein and sterility was performed for 49 days. Results: Nonirradiated and irradiated ALYX RBCs fullfilled all European quality requirements. Regarding final volume and hematocrit ALYX RBCs were less variable than conventionally prepared RBCs. Contrariwise, standard RBCs had a lower supernatant total protein concentration, a higher pH and a higher intracellular ATP concentration throughout storage. Glucose consumption and lactate production showed no differences between the groups. The damage of irradiated ALYX RBCs was reflected in a higher hemolysis rate, a higher extracellular potassium concentration, a higher MCV increase and a faster intracellular ATP decrease compared to nonirradiated units just as in standard RBCs. Conclusions: Regarding red cell quality superior standardisation is the major argument in favor of ALYX RBCs. Some other parameters, e.g. pH and intracellular ATP concentration are advantageous for conventionally prepared RBCs. This may be explained by the use of ACD instead of CPD by ALYX. It is further demonstrated that ACD/SAG-M is a suitable anticoagulant/storage solution for irradiated red cell concentrates despite its slight disadvantages compared to CPD/SAG-M. The decision whether or not to introduce the ALYX component collection system will have to focus mainly on other issues than RBC quality.

PS604

Analysis of Routine Sterile Tubing Welds and New Perspectives

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Background: The German RC BTS West has used sterile tubing welders since 1999. While a great majority of welds showed total integrity, some very small number did not. **Material and Methods:** In 2003 top and bottom blood bag systems of Bag Supplier 1 (BS1) were used in one institute while two others locations used bags of Supplier 2 (BS2) for the preparation of pooled buffy coat derived platelet concentrates (PCs) using the Terumo TSCD® tube welders (Model SC-201 AH). 75% of the connections were filled tube conditions (Wet-Wet). In-process weld integrity was tested using a tube stripping device test procedure. Recently we evaluated as well the new Terumo tube welder TSCD®II (Model SC-203 A). **Results:** 223,955 welds were made in the preparation of 34,971 pooled, filtered PCs. The in-process controls of the welds showed a significant difference between BS1 and BS2, respectively 0.033% failure rate and 0.309%. Those generate a financial loss of approximately 60,000 € per year. The larger number of failures observed in WET - WET conditions for BS2 appeared to be caused primarily by tubing specification (diameter, hardness of the tube and/or wall thickness discrepancies) and tubing combination. After discussing these data with blood bag suppliers and Terumo we found out, that adjusting the devices to BS2 tubes is very difficult, time consuming and costly . Recently Terumo designed a new TSCD®II, and undertook a comprehensive multi-centre study to compare TSCD® with TSCD®II. The initial results of this study confirm that the new TSCD®II device largely eliminates problems related to the differences between tube specification , without the need of special adjustment of the device. **Conclusions:** Tube specifications have great impact on sterile tube connections. As the incidence of failing welds is very low, a large number of controls is required to correctly quantify it. First trials with TSCD®II indicate that it will largely eliminate the requirement for device adjustment to account for tubing differences. To insure satisfactory welding results indifferent tubing combinations (even if both tubing diameters are within the device specification), a thorough validation including air pressure and tensile strength testing is required.

PS605

High Resolution Two Dimensional Gel Electrophoresis and Liquid Chromatography Electro Spray Mass Spectrometry reveal major differences in plasma derived therapeutics

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Purpose: Biological therapeutics are of increasing importance. Plasma and plasma protein concentrates, such as Prothrombin Complex (PPSB) concentrates are widely used in bleeding patients. These products are processed for reduction of pathogen transmission and for purifying clotting factors. The activities of certain clotting factors such as FVIII (plasma) or FIX (PPSB) are the main parameters for quality assessment. However, contaminating proteins, or degraded proteins may also influence the quality of these biological product. These modified proteins may trigger auto-antibodies as it has been shown for erythropoietin, thrombopoietin, and for FVIII concentrates. We used High Resolution Two Dimensional Gel Electrophoresis (2D-PAGE) and Liquid Chromatography Electro Spray Mass Spectrometry (LC-ESI-MS/MS) to assess changes in PPSB concentrates in comparison with normal pool plasma. **Methods:** Three different PPSB concentrates (2 batches each) approved in Germany were used. Following 2D-PAGE, samples were subjected to isoelectric focusing and separated by SDS-PAGE. 2D gels were stained with colloidal coomassie and analysed with the Delta-2D software (Decodon GmbH). Cutted spots were digested with trypsin. LC-ESI-MS/MS analysis was performed on an UltimateTM system (LC Packings) coupled with a Q-TOF mass spectrometer (Applied Biosystems). Database searches employed the SWISSPROT database and a MASCOT search engine (Matrix Science Ltd.). **Results:** Whereas there was reasonable batch to batch consistency, the protein patterns of the three PPSB concentrates varied largely. Beside clotting factors several contaminating proteins such as complement factors (4, 9 and H) and complement H related factor were identified. Differences were determined concerning the content of fibrinogen and antithrombin. **Conclusion:** We provide a powerful new tool to determine protein contaminations and degradation products in plasma derived therapeutics. Further investigations are needed to determine their clinical impact.

PS606

Changes of coagulation activation markers in fresh frozen plasma after introduction of predonation sampling

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Purpose: Introduction of predonation sampling (PDS) was necessarily accompanied by minor changes in the configuration of blood collection systems. In our institution these changes initially went along with an increase of slow-flow donations and premature terminations probably due to clotting processes in the tube system. This prompted us to assess the haemostatic quality of fresh frozen plasma (FFP) by measuring activation markers in the processed plasma. ***Methods:*** Thrombin-antithrombin-complexes (TAT), prothrombin fragment F_{1+2} (both Enzygnost®, Dade Behring) and D-Dimer (DD, Vidas®, BioMerieux) were measured in a total of 128 FFP produced from seven different lots of blood bags of two different manufacturers (A1-A5; B1-B2). Plasma was produced from whole blood donations within 18 hours and was not filtered for leucocyte depletion. ***Results:*** In the first month of PDS use loss of donations increased to 3.4 % from 0.1 % in previous months, mainly due to overtime or premature termination of donations. Elevated values for DD, TAT, F_{1+2} during this period were detected in 10.9%, 23.9% and 21.7% of tested FFP of three different lots (A2-A4, n=46) with the highest values in bags with a gross weight in the lower quintile. Intense investigations of the manufacturer (A) revealed that reduced flow in the aperture of the needle was causative. After amendments of the needle, donation drop outs decreased to the pre-PDS rates. Incidence of elevated DD, TAT and F_{1+2} was markedly reduced to 4.5%, 9.1% and 18.2% (A5, n=22) being almost identical to the incidence in the PDS system of another provider (B2, n=20). Activation markers in FFP processed before PDS (A1, B1, n=40) were comparable to the data obtained in the PDS systems A5 and B2 apart from less elevations of F_{1+2} (5% vs 19%). ***Conclusion:*** Changes of medical devices, even small ones, are always critical. Although the increased incidence of elevated activation markers found in FFPs of one blood bag manufacturer was basically not caused by PDS, another problem coincidentally became evident resulting in modifications of the blood bag's needle. Further investigations are needed to find out the clinical relevance of elevated coagulation activation markers in FFP.

PS607

Proteomics as a tool for quality assurance of blood products

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Purpose: Blood derived therapeutics are increasingly treated to reduce the risk of pathogen transmission. However, recent experience with FVIII concentrates and thrombopoietin indicate that modified proteins can induce autoantibodies with severe clinical consequences. Quantitative and qualitative protein quantification do not allow to identify these modifications sufficiently. We applied High Resolution Two Dimensional Gel Electrophoresis (2D-PAGE) and Fluorescent Two Dimensional gel electrophoresis (DIGE) in combination with mass spectrometry to monitor the protein content and potential modifications of SD treated and pooled plasma and of platelet concentrates. **Methods:** Apheresis platelets and SD-treated plasma were used. A pool of citrated plasma of 72 blood donors served as control. For 2-DE approaches, IPG-strips containing 250 µg protein were subjected to isoelectric focusing (IEF). For DIGE analysis, two platelet protein extracts were first covalently tagged with two distinct fluorescent dyes. A pooled extract of all probes were tagged with a third dye, acting as an internal

standard. Each of the three probes was rehydrated into one IPG-strip, followed by IEF. Second dimension was done by SDS-PAGE. 2D-PAGE gels were stained with colloidal coomassie and analysed with the Delta-2D software package (Decodon). The DIGE-gels were scanned with a fluorescent scanner. For mass spectrometry, proteins were excised from 2D-gels, digested with trypsin and spotted onto the MALDI-targets. MALDI-TOF measurement was carried out on a Proteome-Analyzer 4700 (Applied Biosystems). Database searches employed the SWISSPROT database and a MASCOT search engine (Matrix Science). **Results:** We found changes in the protein patterns of SD plasma as compared to the normal plasma e.g. degraded Inter-alpha-Trypsin-Inhibitor . We also found up to now unknown changes in the platelet proteom during storage. **Conclusion:** Processing of blood products induces changes in the proteom which can be identified by proteomics. Determination of the clinical impact of plasma protein degradation products requires further studies.

PS608

In-vitro study in manufacturing gamma-irradiated LD-RBC units stored > 14 days

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Purpose: TA-GVHD is a rare complication of allogeneic blood transfusion and is caused by immunocompetent donor T lymphocytes proliferating and engrafting in susceptible recipients. The use of gamma-irradiation to inactivate contaminating leukocytes is the key strategy for preventing TA-GVHD. In this in vitro study we evaluated the effects of gamma-irradiation on leukocyte depleted RBC units. National recommendations preferred the irradiation of "fresh" (< 14d) RBC units. With first priority we investigate the feasibility of gamma-irradiation of RBC units after prolonged storage period (until 30 days). **Methods**: Whole blood donations (450ml, n=12) were performed using CPD (63ml) as anticoagulant and SAGM solution (100ml) in a closed quadruple blood container system with integrated soft-shell filter (HGR8437B Baxter). After hard-spin centrifugation the RBC units were immediately leukocyte filtered at 22±2°C and afterwards stored (4±2°C) for different time intervals (7, 14, 21, 30 days). Gamma irradiation was performed using caesium 137 as source (STS device) with a mean dose of 33±2Gy. Immediately before and after the gamma irradiation and after an additional storage of 48h, the biochemistry of erythrocytes and the quality of RBC units were assessed using a hematology analyser (Hb content), blood gas analyser (pH) and photometry (ATP, free hemoglobin and potassium). **Results**: The table summarized the most relevant results (supernatant Hb (mg/L), supernatant potassium (mmol/L) and ATP (µmol/gHb) for RBC units after different storage periods (day 7, 14, 21 and 30) before/after and 48h after gamma-irradiation:

	Supernatant Hb (mg/L)			Supernatant Potassium (mmol/L)			ATP (mol/gHb)		
Day	Pre Rad.	Post Rad.	48h Post Rad.	Pre Rad.	Post Rad.	48h Post Rad.	Pre Rad.	Post Rad.	48h Post Rad.
7	434	389	416	18	18	32	4.28	4.28	4.31
14	451	542	423	26	26	46	5,14	5.14	5.01
21	623	625	537	36	37	49	4,93	4.91	4.67
30	769	648	630	40	40	50	3.51	3.53	3.21

Conclusions: The first objektive of this study was to assess the feasibility of gamma-irradiation on RBC units stored longer than 14 days. We did not observed any significant alterations of RBC units which were irradiated at day 30 and durable for additional 48 hours. The parameters investigated meet the criteria of the national and european requirements. Therefore this gamma-irradiation schedule might be established in the blood bank routine.

PS609

Daily doses of 20 mg of elemental iron compensate for iron loss in regular blood donors: A randomized, double-blind, placebo-controlled study

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Background: A considerable number of regular blood donors develops an iron deficiency, and the exact amount of iron required to compensate for the iron loss from whole blood donation in males and females is still unknown. Therefore the effect of a supplementary balanced diet with ferrous gluconat was examined of the iron status. The diet was added 400 mg vitamin C, in order to increase the resorption of iron. **Study Design and Methods:** A total of 526 regular blood donors (289 male and 237 female) were randomly assigned to treatment with either 40 mg, 20 mg or 0 mg per day of elemental iron as ferrous gluconate for a period of six months, during which one unit of whole blood was collected on four occasions (males) or three occasions (females). Hemoglobin, serum ferritin, and soluble transferrin receptor levels were measured before each donation. **Results:** Daily doses of either 40 mg or 20 mg elemental iron adequately compensated for iron loss in males, who gave blood at two-month intervals, but did not result in a positive iron balance or an increase in storage iron as reflected by the logarithm of the ratio of transferrin receptor to ferritin concentration. In females, who donated at three-month intervals, the same daily doses not only restored the iron balance, but also led to an increase in storage iron. The number of gastrointestinal side effects due to iron supplementation was comparable in both iron groups (11 % or rather 13 %) to the placebo group (11 %). **Conclusion:** The results of this study indicate that 20 mg of elemental iron per day can adequately compensate for iron loss in males and females who donate whole blood up to four (females) or six times per year (males). The supplementary balanced diet was tolerated.

PS610

A feedback interaction system to enhance the quality of blood donation sites run by mobile teams

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Purpose: To increase the efficiency of monitoring quality parameters for mobile blood donation sites, a system of written reporting on a daily basis was developed. **Methods:** A checklist form is completed during each blood donation date by the team personnel, which – in addition to securing basic quality parameters such as hygiene, validation of equipment and securing the criteria for the release of every individual session for blood donation, asks for free formulation of additional comments. The forms and comments are evaluated on the following working day, and a response is generated towards 1) in house team preparation and production department 2) donation date organization department and 3) a timely feedback to the mobile team members who initiated the evaluation and comments. **Results:** We have received a 100% feedback of forms. The evaluation created, in a 9-month monitoring period, a total of 154 localities (out of ca. 400) with suggestions from team personnel. The responses were classified in categories such as hygiene, suitability of area and rooms, donor appearance over time and dynamics of donor flow, room climate, practicability for donation taking, time allotted for setup, acceptance by personnel and teams, and on-site cooperation with Red Cross voluntary personnel. Generally, we achieved problem solutions in ca. 50% of cases within a period of 8 months, especially if repeated reporting on the same issue was recorded for the site in question. Further cases are under monitoring for assessment of their relevance. German Drug Law (AMG) relevant issues were reported in a few cases. The feedback to the team members led to an increase in awareness, interest and motivation within the donation teams, as measured by the frequency and the accuracy with which the comments are made. **Conclusion**: The donation site monitoring system can be successfully implemented and can be adapted to encompass systematic encouragement of personnel by timely feedback of measures taken. In addition to improving quality of blood donation itself, it can be expected to have influence on awareness of team personnel and the overall efficiency and cost-effectiveness of the collection process.

PS611

Controlled implementation of "Predonation Sampling" technique using a quality-assured validation plan

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Purpose: We wished to guarantee quality-controlled and safe implementation of sample deviation ("predonation sampling") in one institute performing ca. 180.000 whole blood collections/year, including controlled release procedures, an optimum of effectiveness and acceptance by personnel and donors, as well as fallback procedures in case of system failures and related security aspects. **Methods:** We prepared a validation plan, including design qualification (DQ), installation qualification (IQ), operation qualification (OQ) and performance qualification (PQ) which covered the preparation, implementation and efficiency of the measure, as well as appropriate failure feedback procedures. **Results:** DQ included the pre-evaluation of donation systems by qualified personnel, and three independent introductory hands-on trainings during normal donation sessions within three months prior to introduction. After quality release of the predonation sample deviation systems and startup in one team 14 days in advance, further 10 donation teams successfully underwent 100% implementation of the new system with one day of supervised use, and repetition and formal release for the new systems on a person-by person basis on the following day (IQ). OQ revealed an overall 2-fold increase (0.25->0.5%) in potentially unsterile products in the month following introduction and a consecutive decrease back to baseline levels. Also, rates of underweight and overweight products increased about 2-4 fold in this period (0.75->2.5%, and 0.1->0.4%, respectively), but returned to pre-implementation values within 4-5 months. Interestingly, the number of products without sufficient laboratory material decreased from 1.5% to 0.5%. PQ showed that component quality was not detectably affected. Bacterial contamination rates were too low to allow conclusions. PQ evaluation of personnel resulted in extra training sessions in selected personnel, and one extra training session for all personnel in handling of balances and avoiding over- and underweight donations. **Conclusions:** The validation plan allowed controlled design, installation, operation and performance of predonation sampling in collection teams. Sample system quality and personnel performance could be held at very low fault levels during the implementation process, resulting in acceptable product loss during implementation and a gain in the overall quality of the collection process.

PS612

Ensuring the quality of red blood cells during long lasting transport on extreme environmental demand

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Background: Apart from military and civilian hospitals across Germany, the blood center of the German Armed Forces in Koblenz guarantees the supply of their field hospitals within international peace-keeping engagement worldwide concerning red blood cells (RBC). Consequently, long lasting transport on extreme surrounding temperature has to be realized without any quality limitation of the drugs compared with the standard defined by German and European authorities. **Material and Methods:** Several commercially available transport box systems of different capacity characterized by either efficient isolation technique, or active electric refrigeration were investigated in a climatic chamber. A couple of packaging protocols were tested and two approaches of temperature measurement (usual inter-product vs. intra-product logger) were examined. The established combined transport by military van and aircraft over two days to Afghanistan was used to confirm the practicability and reliability of the evaluated performance. **Results:** Actively cooled boxes did not appear to be suitable, since no permanent power supply could be ensured and supervised. The isolation box RBC 25 E (Electrolux) containing up to 40 RBC units permitted loading and handling by a single person. A standardized packaging protocol from top to bottom was composed of distinct layers of icepacks (-30°C, the number of which was dependent on the target country's climatic zone), polystyrene, cold storage accumulators (+4°C), knob film, RBC, and identical components without icepacks in reverse order. Conventional temperature logger measuring every 10 minutes facilitated sufficient monitoring compared with an in-product instrument. **Conclusion:** When the presented preparation process is followed, temperature stability can be ensured for at least 42 vs. 64 hours provided that the environmental temperature does not exceed -10°C or +40°C, respectively. Additional parameters, such as temperature increase by sun radiation, damage of the erythrocytes by air pressure change, or effects of vibration or humidity on the cells' stability have to be validated to further optimize the transport conditions in terms of the blood products' therapeutic quality.

PS7: Cellular and Immunotherapy

PS701

Human platelets target dendritic cell function by decreasing bioactive IL-12 secretion

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Background: Dendritic cells (DCs) are the most potent antigen-presenting cells that initiate and regulate immune responses. They are unique in their feature to produce bioactive IL12, a major pro-inflammatory cytokine which promotes expansion of T helper 1 cells and NK cells, connecting innate and adaptive immunity. Platelets are highly reactive components of the circulatory system with well-documented hemostatic function. Recent observations extend functions of human platelet derived products such as platelet activating factor 4 (PF4), histamine, sphingosine-1-phosphate (S1P), lysophosphatidic acid (LPA) and CD40L as immunomodulatoric. However, limited data are available on the effect of intact human platelets on DC differentiation, maturation, and function. **Study design:** Magnetic-bead sorted CD14+ cells were differentiated to DCs in the presence of syngeneic platelets or Thrombin-Receptor-Agonist (TRAP)-activated platelets from healthy donors. DC differentiation, maturation and function were investigated relative to control DCs expanded without platelets. **Results:** Platelets did not affect DC differentiation or maturation, as demonstrated by comparable CD1a, CD14, CD40, CD83, CD86, and HLA-class-II expression. In contrast, platelets affected DC function by impairing 41% of TNF-alpha production and 50% of bioactive IL-12 production. If DCs were developed in the presence of TRAP-activated platelets, their secretion of IL-12 and TNF-alpha was also diminished, but to higher extend. Interestingly, production of the immunosuppressive cytokine IL-10 seemed to be lifted in coculture with TRAP-activated platelets. To explore the nature of the platelet-derived factor inhibiting IL-12 production, trans-well experiments were performed. These data revealed IL-12 reduction even if cell-cell contact was avoided, suggesting platelet-derived soluble factors responsible for this effect. **Conclusion:** These results indicate that platelets suppress activation of human monocyte-derived DC by decreasing bioactive IL12 secretion in a cell-to-cell contact independent manner. The immunomodulatory interaction of intact platelets with DC is of prime importance for clinical DC therapy.

PS702

Profound sex differences in TLR 7-induced IFN-α production by plasmacytoid dendritic cells through imidazoquinolones

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Purpose: Interferon-α (IFN-α) is a critical cytokine inhibiting virus-replication and cell proliferation and has been suggested to be involved in the pathogenesis of systemic lupus erythematodes (SLE). Plasmacytoid dendritic cells (pDCs) represent the principal producers of IFN-α in human blood after stimulation through Toll-like receptors 7 (TLR7) and 9 (TLR9). With respect to the important role of TLR-ligands as novel immune adjuvants and the strongly increased frequency of female subjects suffering from SLE, we analysed the inter-individual differences in TLR-induced IFN-α production in human blood donors. **Methods**: Blood samples of two prospectively collected groups (n1=100, n2=120) of healthy individuals were stimulated in a highly standardized manner with Imidazoquinolones (TLR7 ligand) and CPG ODN (TLR9 ligand). IFN-α protein was measured by ELISA. **Results**: TLR7 stimulation revealed a profound sex difference between female and

male pDCs to produce IFN-α. Female subjects consistently produced 3-4 fold higher IFN-α levels when compared to male individuals (mean female versus male: 182 vs. 55 pg/ml; p<0.0001, n1=100). In contrast, no gender difference was observed after TLR9 stimulation indicating that signalling downstream of TLR is unlikely to cause these differences. The findings were confirmed in a second, independent group of individuals (n2=120, p<0.0001). **Conclusion**: Our data reveal a profound TLR7- and gender-dependent pathway of IFN-α induction in human pDCs. Genetic dissection of this pathway may help to understand the high female prevalence in SLE and other autoimmune diseases and is of importance with respect to the clinical utilization of synthetic TLR7 ligands.

PS703

Profound deficiency of circulating dendritic cell subsets in kidney transplant recipients on steady-state immunosuppressive therapy: Preliminary results of a prospective match-controlled study

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Purpose: Traditionally, lymphocytes are considered the principal cellular target of immunosuppressive agents. However, recent evidence from in vitro studies suggests that immunosuppressive drugs interfere with immune responses at the earliest stages by suppressing dendritic cells (DCs). DCs are professional antigen presenting cells that initiate and regulate immune responses. The purpose of this ongoing study is to analyze the in vivo impact of long-term immunosuppression on circulating DC subsets after kidney transplantation. **Methods:** 46 kidney transplant recipients on immunosuppressive therapy with different agents (cyclosporine, tacrolimus azathioprine, MMF, prednisolone) were included and compared with 46 age- and sex-matched healthy controls. Three major DC precursor subsets: Myeloid DCs1 (mDC1) and 2 (mDC2) and plasmacytoid DCs (pDC) were identified by four colour flow cytometry according to lack of surface CD14 and CD19 and positive expression of CD1c, BDCA-2 and BDCA-3. **Results**: Long-term immunosuppression resulted in significant reduction of all major DC subsets in comparison to healthy controls (mDC1 p<0.001; mDC2 p<0.0001; pDC p<0.00001, two-tailed Mann-Whitney Test) with the strongest impact on pDC numbers (5073/ml vs. 10590/ml in healthy controls). In contrast, total leukocyte numbers were not significantly affected. Since corticosteroids have been suggested to have a major impact on DC numbers, we stratified patients depending on prednisolone medication. This analysis revealed a significant negative impact of corticosteroids on all DC subsets. Importantly, pDC and mDC2 numbers were still significantly decreased in patients with steroid-free immunosuppression compared to healthy controls (mDC2 p<0.003; pDC p<0.0005), whereas total leukocyte counts again were not significantly altered. **Conclusion:** Circulating DC subsets are significantly reduced in patients on steady-state immunosuppressive therapy. The major DC subsets affected are plasmacytoid DCs (pDCs) and the minor myeloid DC subset mDC2. We propose that classical immunosuppressive agents are likely to have a strong negative impact on DCs besides their well-known affects on lymphocytes.

PS704

Topical use of autologous platelet concentrate in maxillofacial surgery. A prospective trial

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Purpose: Alveolar bone regeneration is frequently necessary for dental implant placement in maxillofacial surgery. In order to enhance wound healing and formation of new bone, attempts were made to increase peptide growth factors by the use of topically administered autologous platelets. We conducted a clinical, bicenter, randomized, prospective and controlled trial to prove the effect of platelets on wound healing and bone grafting in patients undergoing maxillofacial procedures. **Methods**: The trial has been set up two years ago as a two-center-study in Giessen and Erlangen. Included were 50 patients who needed osteoplastic bone graft from the iliac crest for sinusfloor augmentation prior to implant placement. Autologous platelet concentrate (APC) was obtained from platelet rich plasma (PRP) derived from 450 ml of CPD anticoagulated blood. After storage for 24 hours, PRP was concentrated by different centrifugation steps and adjusted to 10^{10} platelets per ml. Platelet gel was generated intraoperatively from 3 ml platelet suspension by the use of 0,5 ml 10% calcium gluconat and 0,5 ml autologous native blood (thrombin). This mixture was added to approximatly 3 cm^2 autologous bone graft. The platelet gel and bone preparation was performed by using autologous materials alone. Operation procedure is done in every patient group by standardized protocol for bone grafting in sinus floor augmentation. The intervention group was additionally treated intraoperatively with local application of APC. Panoramic radiographics, computerized tomography and bone biopsy were taken 4 months later. **Results:** The recruitment of the study has already completed, so that first results can be presented. One patient in the APC group showed localized resorption of the bone graft and one patient must be treated because of a sinusitis maxillaris. Histological evaluation of biopsy revealed numerous areas of osteoid and bone formation without evidence of inflammatory cell infiltrate. The bone volume reached from 40-70%. The final bone graft controlled by CT showed remarkably dense bone in sufficient quantity. **Conclusion**: Based on the preliminary data of this study topical use of APC might be efficient to improve osteoid bone formation in the maxilla prior to dental implant placement and regarded as a promising therapeutic procedure.

PS705

Platelets and Apoptosis – the Hidden Face of Wound Healing

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PURPOSE: Previous reports demonstrate that platelets induce apoptosis in various cells and sphingolipids seem to be involved in this platelet-cell interaction. In respect to wound healing, apoptosis is an important energy saving mechanism to eliminate superfluous cells or cells with completed mission from the wound space. We now tested platelets and platelet compartments (membranes and releasate) for their ability to induce apoptosis in fibroblasts and osteoblasts. In order to further elucidate the underlying mechanism we used specific inhibitors of sphingosine metabolism. **METHODS**: Primary human fibroblasts and osteoblasts were cultured under defined standard conditions. Apoptosis was measured by either a commercial flow cytrometric assay on the basis of TdT-mediated fluorescein-dUTP nick end-labeling (TUNEL) method which labels the 3´-OH ends of DNA fragments generated by apoptosis, or by an ELISA measuring cytosolic BrdU-labeled DNA fragments. Caspase-3, -8 and-9 activities were measured by a colorimetric assay based on their recognition of specific amino acid sequences. **RESULTS**: In contrast to osteoblasts, apoptosis was induced by isolated platelets in fibroblasts only. This activity was related to platelet membranes, whereas platelet releasate had no effect. Inhibition of glucosylceramide synthase by PPMP (1-phenyl-2-palmitoylamino-3-morpholino-1-propanol) abolished platelet membrane-induced apoptosis and induced caspase-9 activity, whereas inhibition of ceramide synthase by fumonisin B1 enhanced apoptosis and induced caspase-8 activity. This indicates that ceramide generation seems not to be the major pathway for platelet-induced apoptosis rather than induction or enhancement of death receptor pathways by sphingosine. This was confirmed when platelet membranes enhanced TRAIL- and Fas-induced apoptosis. **CONCLUSION**: Our results confirm the ability of platelets to induce apoptosis. Bioactive sphingolipids derived from platelet membranes seem to be involved as a trigger or enhancer of death receptor pathways. This effect may have relevance in tissue repair when activated platelet membranes are deposited in the fibrin clot and surrounding cells are growing in this provisional matrix. Osteoblasts seem to be less affected by platelet-induced apoptosis due to the absence of TRAIL receptor.

PS706

Platelets Induce Proliferation and Maintain the differential state of Human Osteoblastic Cells

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PURPOSE: Platelets are a rich source of autologous growth factors with the potential capacity of accelerating wound healing and tissue regeneration. In contrast to the extensive knowledge of single growth factors, the entire effect of the mixture released by the platelets are poorly understood. As the clinical application of platelet preparations in bone regeneration gains in importance, we studied the effects in cell culture with osteoblasts and osteoblast-like-cells (SaOs) in monolayers and in three-dimensional plasma gels. Furthermore, we showed the changes of mRNA expression of CBFA1, a marker of osteoblast differentiation, and collagen type I in cells stimulated with platelets. **METHODS and RESULTS**: In proliferation assays, platelets induced proliferation with maintained expression of alkaline phosphatase in SaOs Cells. The effect was induced by both, activated platelet releasate and membranes. Von Kossa staining of long-term cultured osteoblasts showed an increased deposit of extracellular calcification in platelet-stimulated cells and the activity of alkaline phosphatase was not reduced compared to untreated cells. In three-dimensional plasma gels, a model for the clinical application of concentrated platelet-rich-plasma (cPRP), supplementation of platelets to the gel strongly increased cell migration of osteoblasts into this provisional matrix. In this model, proliferation was even more pronounced compared to the monolayer. Furthermore, cryosections and immunofluorescence of the plasma gels showed a maintained expression of collagen I and bone sialoprotein. Additionally, RT-PCR analysis of platelet-stimulated osteoblasts showed an increased expression of CBFA-1 and consistent collagen I mRNA. **CONCLUSION:** This study clearly demonstrates the known proliferative effects of platelets on osteoblastic cells. Stimulation by platelets has the exceptional effect to induce expansion of the cells with maintained differentiation. This implicates the clinical application of autologous platelet preparations for improved wound healing and tissue regeneration.

PS707

Differentiation of dendritic cells under the influence of platelets

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PURPOSE: For the clinical use of dendritic cells (DC) as cancer vaccine, a large amount of monocytes are needed to generate DC. Monocytapheresis has been established as a standard procedure to collect sufficient monocyte number for the complete vaccination series. With regard to the content of contaminating platelets (Plt) within the final monocytapheresis product, a high concentration of Plt could be found. It is of interest to find out whether these Plt interact with monocytes (MO) respective to their differentiation to DC. The aim of this study was to investigate the DC differentiation under influence of Plt with regard to function and phenotype. **METHODS:** MO (CD14-positive cells) were positive selected by magnetic cell sorting. From the same donors platelet-rich-plasma (PRP) and plasma were obtained. The purity of selected MO was over 95%. Subsequently, MO were incubated with plasma or Plt in different concentration (1 MO/5Plt , 1MO/15Plt, 1 MO/45Plt). MO alone served as negative control. After this step, MO were cultured with IL-4, GM-CSF and maturation cytokines (IL-1ß, IL-6, TNF-α and PGE_2) to fully mature DCs. On day 8, tetanus toxoid was added to immature DC. The ability of immature DCs to capture soluble antigen was analysed using FITC-dextran and flow cytometric analysis. According to T cell stimulation, mature DCs were subsequently co-cultured with autologue T-cells. Stimulated T-cells were analysed for activation and proliferation as well as for intracellular cytokines. **RESULTS:** The yield of MO-alone-DC, MO-plasma DC, MO-5Plt DC, MO-15Plt-DC and MO-45Plt-DC was 19.4 ± 7.6 %, 12.3 ± 3.2 %, 11.8 ± 2.6 %, 11 ± 2.7 %, 11.2 ± 3.8 %, respectively. No significance in the capture of FITC-dextran in all DC-cell lines could be observed. All DC-cell lines were negative for CD14. Respective to the expression of co-stimulatory molecules on mature DC, with Plt cultured DC showed higher values for HLA-DR, CD80, CD86. The most expression of these markers was observed on DC differentiated from MO contaminated with 5 and 15 Plt. Accordingly, the maximal T-cell proliferation and activation as well as cytokine production of stimulated T-cells was also recorded with regard to the stimulation by these DC-cell lines. **CONCLUSION:** Our results indicate that Plt have no effects on MO differentiation to DC. However, Plt enhance the expression of co-stimulatory molecules on DC. Accordingly, these DC strongly stimulate T-cells with regard to their proliferation and activation. These insights might be helpful for the use of DC in the therapy of cancer diseases.

PS708

Magnetic labelling of Stem cells with Fe_3O_4 containing Nanoparticles

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Purpose: Stem cells have attracted a lot of interest as it has been shown that they can repair organ structures and functions in different rodent models. Migration and homing of these cells to the site of repair has become an important aspect. One possibility of tracing is the use of magnetic nanoparticles which can be detected by nuclear magnetic resonance (NMR). **Methods:** We used two commercially available magnetic nanoparticles (Feridex and Resovist) for studies with mesenchymal stem cells (MSCs), HeLa cells and CD34+ cells. We incubated these cells with the nanoparticles (typically 25 µg Fe/ml) with or without 1,25 µg poly-L-lysine (PLL)/ml for 24 h. The cells were then washed and histochemically stained by the Prussian blue reaction. Cells containing iron particles were further digested in a mixture of perchloric acid and nitric acid. The amount of iron uptake was determined by a ferozine based method. Detection of cell suspensions in 5% gelatin was performed in a clinically used NMR scanner. **Results**: Prussian blue staining showed uptake of Feridex and Resovist when they were combined with PLL in MSCs. MSCs contained typically 15 to 20 pg/cell. If Resovist or Feridex was used without PLL, only in MSCs incubated with Resovist a detectable amount of iron was noted in the Prussian blue staining (about 2 pg Fe/cell). By increasing the concentration of Resovist in the medium (250 µg Fe/ml) MSCs were labeled with 10-12 pg Fe/cell. By electron microscopy we showed that the iron particles were contained within cellular compartments. In order to achieve a 50% reduction of the T2 signal of 5% gelatin, 250.000 cells/ml (labeled with Feridex or Resovist and PLL, containing 10-12 pg Fe/cell) were needed. Similar results were obtained with HeLa cells. Uptake of magnetic nanoparticles in CD34+ cells was not detected by Prussian blue staining and others have reported values of around 2 pg Fe/cell. Because the most striking difference between these two magnetic nanoparticles is the type of dextran (dextran versus carboxydextran) we have started to synthesize nanoparticles with different amounts of carboxylic side groups. **Conclusions:** In order to label cells for detection by NMR Feridex and Resovist can be used in conjunction with PLL. Only Resovist shows a significant uptake of iron particles in MSCs and HeLa cells without the need for a transfecting agent. Efficacy of labeling CD34+ cells is much lower. Magnetic labeling may be used particularly for monitoring of migrations of MSCs and tissue regeneration studies. Labeling techniques of CD34+ cells needs to improve before it can be used in a clinical setting.

PS709

Clinical Grade Expansion of the Natural Killer Cell Line NK-92 – Evaluation of Life Cell® Culture Bags versus Nunc Cell Factory®

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Background: NK-92 cells are an immortalized cell line that exhibit non-MHC restricted anticancer activity and hold the promise to be developed for non-directed, "ready to use" adoptive immunotherapy. Here we report about our concept of expansion of NK-92 cells from a master- and working cell bank, respectively. Moreover, we have evaluated two different culture con-

tainers (Life Cell tissue culture bag, Baxter; Cell factory, Nunc) and compare these with regard to the growth characteristics and functional activities of clinical scale expanded NK-92 cells. **Methods:** Starting from a working cell bank, a population of NK-92 cells is maintained in culture bags at a concentration of $2x10^4$ and $5x10^5$ NK-92 cells/ml X-Vivo 10 medium (Cambrex, Belgium) supplemented with either 100 or 500 IE/ml IL-2 (Proleukin®) and 5% heat inactivated human plasma (NK-92 medium). For expansion, NK-92 cells are seeded at a concentration of $2x10^4$ into either culture bags (1700ml NK-92 medium) or Nunc Cell factory® (2000ml NK-92 medium) and grown in batch culture until the time of harvest. Every two days, samples are drawn and the cell number and viability assessed using trypan blue counting. At the point of highest cell density (usually 12-14 days) the cytotoxicity was assessed using a flow cytometric assay and the NK-sensitive cell line K562. **Results:** The maximum density of NK-92 turned out to be highly dependent on the IL-2 concentration provided with the media. In X-Vivo 10 supplemented with 100 IE/ml the maximum density was $4x10^5$ cells/ml (culture bag) and $1x10^6$ cells/ml (Cell factory), whereas 500 IE/ml IL-2 mounted NK-92 cell densities of $1,1x10^6$/ml (culture bag) and $2,1x10^6$/ml (Cell Factory), respectively. The doubling time of NK-92 cells was comparable in both culture systems with 36hrs. The clinical scale expansion in culture bags only moderately impaired the cytotoxic activity of NK-92 which was 54% lysis of K562 compared to 65% in Cell Factory expanded cells. **Conclusion:** Both systems, culture bags and Cell Factory allow the clinical scale expansion of NK-92 cells without significant impairment of NK cell function. Albeit the somewhat lower cell density achieved with the culture bags, the closed system offers substantial advantages when compared to the "open" and laborious Cell Factories. To expand NK-92 cells to a clinical applicable dosis of $5x10^9$ NK-92/m^2 body surface 5 bags with a total of 10l medium are needed. Starting from a stock culture bag it takes 10 days for expansion.

PS710

Establishement of an external UV-A irradiation system in combination with a conventional cell separator as an alternative and feasible technique for extracorporeal photopheresis

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Background: Extracorporeal photopheresis (ECP) is FDA approved for treatment of cutaneous T cell lymphomas and has been found to be effective in the therapy of steroid-refractory graft-versus-host-disease as well as several autoimmune diseases. The purpose of the present study was to develop an external UV-A irradiation system for ECP that can be easily combined with several cell separators. **Methods:** Together with an external manufacturer we established a CE-certified UV-A irradiation chamber that allows dose- (J/cm2) and wave-length controlled UV-A irradiation of blood products. Mononuclear cells were collected with the MCS plus Haemonetics cell separator.**Results and Conclusion:** The combination of an external UVA irradiation chamber with a conventional cell separator proved to be a feasible, safe and highly cost-effective alternative to the current commercial systems available. Especially the combination with a single-needle discontinous cell separator was found to be very suitable and feasible for patients with difficult venous access. So far we have performed more than 100 photopheresis procedures with 6 patients suffering from either cutaneous T cell lymphoma or steroid-refractory GVHD.

PS711

Selective cell apheresis: A new approach in the treatment of active inflammatory bowel disease

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Background: Active inflammatory bowel disease (IBD) is often associated with elevated and activated monocytes/macrophages and granulocytes, which are the main source of inflammatory cytokines. Therefore they may excert a prominent role in initiation and perpetuation of IBD. Thus several studies have indicated that leukocytapheresis is an useful option in patients after failure to conventional therapy. Here we evaluated a new aphersis system (Adacolumn, Otzuka, Japan) which adsorbs selective activated monocytes, macrophages and granulocytes out of the patient's whole blood by columns containing cellulose acetate beads. **Patient and Methods:** Patients (n = 7) suffering from colitis ulcerosa were scheduled to Adacolumn treatment (ADA) after failure to conventional treatment. All patients received 5 ASA (sulfasalacin) and steroids during ADA. Apheresis procedure was done once a week for five weeks. Follow ups were done every two weeks after completion of ADA. Treatment respond was documented by disease activity index (CAI), CRP levels and steroid demand before ADA (week 0) and every 2 weeks after ADA until week 12. **Results:** One patient was rejected from analysis because of acute exacerbation of underlying disease immediately after ADA. 2 patients showed a transient response. In 4 patients a marked overall improvement was observed. The median demand of steroids decreased from 20 mg/d (5-20) to 5 mg/d (0-12.5) and the CAI fell from median 8.5 (0 -14) to 6.5 (0 -14) points. CRP values remained almost unchanged (0.6, range 0.5 -3.7) week 0 vs 0.6 (0.5 -1.69) week, respectively). **Conclusion:** Our results show that selective adsorption of activated monocytes/macrophages and granulocytes has a positiv effect on IBD and is an useful option in patients failing to other therapy regimens.

PS712

Maintenance of life-saving neutrophil functions up to 72 hours *in vitro*

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Purpose: Severe neutropenia, i.e. 500 or less neutrophil granulocytes (NE) per µl blood, represents an indication for supportive transfusion of NE but particularly due to NE short shelf-life this therapy lacks acceptance. In this study we made a new attempt to extend NE shelf-life by compounding NE concentrates with three different storage additives. **Methods:** In 18 healthy donors NE were mobilized by application of rHuG-CSF (lenograstim, 5 - 10 µg per kg body weight, s.c.) in combination with dexamethasone (8 mg, orally) 8 to 16 h prior to apheresis. The apheresis product volume averaged 203 ± 8.7 ml containing $8.3 \pm 1.6 \times 10^{10}$ NE. NE were stored undiluted (1, n = 18) and diluted 1-in-2, -4, -8 using T-Sol, a platelet additive solution (2, n = 6), T-Sol + 1 % human serum albumine (HSA) (3, n = 6), or autologous plasma (4, n = 6) for 72 h. Hemograms, pH-values, phagocytosis, oxidative burst, and interleukin (IL)-1β-, IL-8-, tumor necrosis factor (TNF)-α-levels were assessed in 24 h intervals. **Results:** Due to sample withdrawal and definitely to some extend autolysis NE-count decreased from $4.3 \pm 0.8 \times 10^{10}$ to $2.2 \pm 1.0 \times 10^{10}$, and pH-value dropped from 6.35 ± 0.34 to 5.37 ± 0.15 within 72 h (1), whereas 1-in-4, -8 dilutions exhibited more consistent hemograms and pH-values above 6.0. 1-in-8 dilution (4) stabilized pH at 7.09 ± 0.36 after 72 h. Function deteriorated to about 50 % within 24 h (1), but 1-in-8 (3), and 1-in-4, -8 diluted NE (4) exhibited functional performance above 90 % still after 72 h storage. In all collectives cytokine levels increased over time. After all, IL-1β ranged between 31.0 ± 16.3 (1-in-4, 4) and 100.0 ± 21.4 (1-in-4, 2), IL-8 from 513 ± 454 (1) to 3180 ± 760 (1-in-4, 2), and TNF-α between 3.8 ± 1.7(1-in-2, 2) and 23.2 ± 11.8 (1-in-8, 4) [pg/ml]. **Conclusion:** NE apheresates may be half-transfused instantaneously and for subsequent therapy half-diluted 1-in-8 with -Sol + 1 % HSA or 1-in-4, -8 with autologous plasma and stored up to 72 h. Confirmatory trials will have to prove the usefulness of our promising in vitro results for effective neutropenia therapies in vivo.

SY1: The Future of Blood Grouping: Mass Genotyping for Blood Groups and Beyond

SY100

Introduction: The future of blood grouping is mass genotyping

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Blood grouping has always been done by serologic, i.e. antibody based, methods. More than 15 million blood donations are typed for ABO and D each year by such methods in Europe. Similar numbers apply to patient testing which has to cover additional blood group systems and is often technically more demanding. Since 1990 the genetic bases of the blood groups have been established. The allele polymorphism was recognized to be more complex than anticipated by many transfusion medicine specialists. However, within a mere 10 years all basic genetic research issues have been resolved. Experienced serologists have somewhat grudgingly accepted the support by blood group genotyping which can bridge certain diagnostic gaps, but they continue to maintain that genotyping may only be useful to complement serological data. The application of molecular techniques and the exploration of the molecular basis were instrumental for almost all recent significant contributions to the basic and clinical aspects of blood groups. Major progress in elucidating serologic conundrums was made by the molecular exploration of questions that escaped for decades their characterization. Other results contributed considerably to basic biologic research and exemplified the relevance of blood group research beyond medicine. A major part of the future of blood group typing will clearly rely on genotyping. In this context, this symposium explores the options for mass genotyping. It does so by addressing the current knowledge of the molecular bases; the specific applications that are used routinely or may become routine shortly; the current applications in two European countries; the ethical issues of mass genotyping; and the industry's perspective taking into account the experience from previous similar technology changes.

SY101

The BloodGen Project: Platform technology for mass scale genotyping of blood groups and beyond

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Purpose: Presently molecular genotyping of blood group is confined to a limited number of patient groups including fetuses and multi-transfused patients, where serology is difficult and possibly harmful to the patient. If applied on a large scale, genotyping may represent a viable alternative to red cell serology for routine donor and patient blood grouping. Accurate genotypic assignment may lead to a reduction in the incidence of alloimmunisation and perhaps eventual elimination of serological cross matching before transfusion. The BloodGen consortium was assembled to test the feasibility of molecular genotypic assignment of blood group status in European donors. **Methods:** Oligonucleotide arrays and fluorescent based PCR amplification systems have been developed by BloodGen consortium members. The array contains 4,608 spots corresponding to 94 SNPs that are clinically significant blood group alleles. The chip is capable of genotyping the major alleles of the ABO, RH, KEL, FY, JK, MNS, DI, CO and DO systems on a single slide format. The array is hybridised using multiplex Cy3-labelled and fragmented PCR products amplified from donor DNA, washed using an automated hybridisation station, scanned and genotyped using software undergoing development by the BloodGen partner Progenika. **Results**: A prototype BloodGen array and fluoro-SSP assay has been produced. The prototype array has correctly assigned blood group genotype of most of the 94 SNPs assembled, and requires optimisation to improve signals of some. These modifications will result in the production version of the BloodGen array, to be made commercially available at the conclusion of the project in 2007. The production array will be screened by BloodGen consortium members in the latter stages of the project using a large cohort of donor DNA samples collected from all ethnic groups found in Europe. **Conclusions:** For blood group genotyping to replace serological testing, a product that is 100 % reliable will need to be field-tested against a large cohort of donor DNA samples. The BloodGen consortium will prove the validity of two different platform technologies, and will result in the commercialisation of two viable products at the conclusion of the BloodGen project. Large-scale implementation of blood group genotyping may eliminate alloimmunisation to frequently untested blood group antigen incompatibility (e.g. Jk^a antigen) by identifying those donors that have clinically significant low frequency alleles.

SY102

Molecular genetics of blood groups (excluding Rh): From genotype to phenotype

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Background: Over the past two decades, the molecular genetic bases for all but one of the 29 blood group systems have been characterised and the underlying nucleotide differences for all clinically important antigens have been identified. Not surprisingly, many complementary pairs of antigens are encoded by single nucleotide polymorphisms (SNPs), but there are antigens and other more complex phenotypes the are the products of nucleic acid exchange between closely related genes. Null phenotypes arise from many different backgrounds, from nonsense SNPs to deletion of the entire gene. In the ABO blood system, we, and others, have shown that weak A or B antigen expression can result from missense SNPS that encode for a less efficient glycosyltransferase; or more interestingly, from the formation of hybrid genes. In the Kidd blood group system, we have defined the genetic background for the more common Finnish and Polynesian Jk_{null} phenotypes. **Methods:** We have developed a procedure that employs different allele-specific polymerase chain reaction (PCR) assays for the rapid detection of simple and complex *ABO* alleles. Similarly, we have developed allele-specific PCR assays for the detection of common and rare *JK* alleles. All assays have been validated for use on genomic DNA derived from whole blood. Samples tested have been referred to the Nordic Reference Laboratory for Blood Group Genotyping for identification. **Results:** We have identified novel point mutations and hybrid genes responsible for the weakened expression of A and B antigen. Examples include the A_x phenotype that can result from a SNP in the consensus A^1 allele or from a $B\text{-}O^{1v}$ hybrid gene. The allele-specific PCR assay for *JK* allele detection has been used for routine genotyping analysis as well as the detection and confirmation of referred Jk_{null} samples. **Discussion:** The ABO and JK assays can be used independently for the analysis of serologically discrepant samples or maybe incorporated into a more complete blood group genotype analysis of patient or donor samples. These two systems provide a model for the investigation of blood group genes and for the accurate prediction of phenotype.

SY103

Molecular genetics: The two *Rhesus* genes and their *Rhesus* boxes

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The RH blood group system is the most complex blood group system. Its serologic complexity includes more than 40 different antigens and more than 30 D epitopes and is caused by more than 100 known *RH* alleles. The antigens of the RH blood group system are located on two Rh proteins: RhD is expressing antigen D and RhCE is expressing antigens C, c, E or e. The *RHD* and *RHCE* genes are composed of 10 exons distributed along ~60,000 bp genomic sequence each, have opposite orientation, and are only 30,000 bp apart. The *RHD* gene is flanked by two homologous DNA segments of about 9,000 bp dubbed *Rhesus boxes*. RhD has 6 exofacial loops, three of which differ from their RhCE counterparts and form the antigen D. Gene conversions or missense mutations causing amino acid substitutions in exofacial protein segments often lead to the expression of low frequency antigens or to the lack of distinct epitopes in partial D; amino acid substitutions in trans-

membraneous or intracellular protein segments usually lead to a weak D phenotype with weakened antigen expression but no prominent antigen alteration. The D negative phenotype is caused by the absence of all RhD specific exofacial protein segments, most frequently due to a deletion of the whole *RHD* gene that occurred by a recombination of the *Rhesus boxes*. The recombinant "hybrid" *Rhesus box* may be detected by PCR, an approach often used for *RHD* zygosity determination. The main antigens encoded by *RHCE* are largely determined by amino acid polymorphism at position 103 (C/c) and 226 (E/e) but also depend on the general structure of the Rh protein. The phenotypic variation of RhCE largely parallels that of RhD with the major difference that there is no frequent *RHCE* deletion. The complexity of the *RH* genes is a challenge for antigen prediction by genotyping. D antigen prediction is confounded by *RHD* positive, D negative alleles that must be distinguished from normal RhD. For *RHCE*, even seemingly simple tasks like a correct prediction of the antigens C, e or f turned out to be surprisingly difficult. The complexity of the *Rhesus boxes* in Africans is only emerging today and needs to be further investigated to enable a correct *RHD* zygosity testing in Africans.

SY104

Clinical benefit for the community: Ethical issues and opportunities involved in mass genotyping of blood groups

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The clinical benefit is addressed in all contributions to this symposium. In several clinical situations blood group genotyping has already been established to improve patient care. Examples include testing of fetus from amniotic fluid; of multiple transfused patients and of patients with auto- or alloimmunohemolytic anemia if standard serology fails; of fathers for a *RHD* heterozygous status; and of serologic D neg. donors for the inadvertent presence of weak D or chimerical blood. Several genotyping applications have resulted in or will likely contribute to cost containment efforts. They include the specific detection of weak D types to avoid anti-D prophylaxis and to reduce use of D neg. blood units for those patients who do not need them. Another major application would comprise the testing for fetal *RHD* genotype during pregnancy from maternal plasma to obviate the need of anti-D prophylaxis in about 40 % of all pregnancies in D neg. mothers; this approach may become implemented in the near future as detailed in a subsequent abstract. Once genotyping can be performed at an individual blood donor basis, it is quite obvious that such an approach may serve as a platform technology. Then economies of scale may become effective, because huge numbers of ABO confirmatory typing is required for blood donors each year. The technology must not be restricted to blood groups but might also cover for instance the viral nucleic acid testing. The experience established in transfusion medicine may be transferable to other kinds of genotyping approaches. The public is rightfully concerned about possible negative effects following the advent of mass scale genotyping. However, the application of mass scale genotyping for blood groups is special compared to many other kinds of genotyping, because no adverse ethical issues exist and it should hence be easy to resolve legal issues, if any remain. In particular, transfusion medicine specialists can determine the clinical implications of genotyping results in all cases by referring to standard immunohematology results.

SY105

The European perspective: Current genotyping in the Czech Republic

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Genotyping for blood group antigens is performed in the Czech Republic from 1996 when the exon-scanning PCR-SSP for RHD described by Ch.Gassner was introduced in Reference Laboratory for Immunohaematology, Institute of Haematology and Blood Transfusion in Prague in cooperation with University of Innsbruck. Later this technique was applied for genotyping of other blood group systems (ABO, Kell, Kidd, Duffy, MNSs, Rhesus system for detecting RHCE and RHD weak and variant alleles, HPA) using commercialized PCR-SSP kits produced by INNOTRAIN. In 2004 we have started to use PCR-SSP kits produced by BAG. More than one thousand of samples were genotyped so far. Indications for genotyping are: serological discrepancies, multitransfused patients, cases of erythrocyte and thrombocyte feto-maternal incompatibility, weak or suspect variant RhD in pregnant women (for answering question about need of anti-D prophylactic administration). In 2003 the real-time PCR for RHD and RHCE alleles was introduced in the Faculty Hospital Motol in Prague and was used for fetal genotyping from maternal plasma. The most frequently detected RHD variants in our population are: D VI (prevalent is type 1, no case of type 3 or 4 detected so far), DFR and D VII. We have detected three unrelated cases of DCS variant (RHD-CE/partial exon 5 exchange/-D, so far found in our population only). Other rare cases were: D IIIc, D IV type 4, DNB, DOL (donor with Jordanian ancestry), DYO (Corean patient). Regarding weak D alleles the most frequent in Czech population is type 1 (many cases, twice more than type 3, with only several cases of other types – 2, 4, 5). We have found the GATA box Duffy null allele in a relatively high frequency in the Gipsy population living in the Czech and Slovak Republic. Our laboratory is taking part in the EU BloodGen project – genotyping using microarray technology. We hope that this project will bring more knowledge about blood group alleles distribution in our population.

SY106

The European perspective: Current genotyping in Spain

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Purpose: Current genotyping in Spain is mostly applied to those cases in which the antigen determination is difficult or not possible. However, genotyping methods are foreseen as a valuable alternative to serological methods and strategies for other applications, like high-throughput blood group typing are being developed. **Methods:** Blood group genotyping in Spain is performed in Reference Laboratories and includes:

- *RHD* genotyping of donors with an anomalous D antigen expression
- *ABO* genotyping of donors with a discrepancy in the forward and reverse grouping.
- *HPA-1* typing to identify HPA-1a negative donors

It is also used in blood group typing of red cell panel donors:

- For Fy^x allele confirmation in donors with weak Fy^b expression
- For searching and confirming unfrequent phenotypes

Occasionally, it is also applied to blood group typing of patients that have received multiple transfusions. Blood group genotyping is also used in the context of pregnancy, to determine feto-maternal incompatibility in sensitized pregnant women:

- Fetal blood group typing from amniotic fluid samples is available for *RHD, RHCE,* KEL, JK and FY.
- More recently, fetal RhD status can be determined from the cell-free DNA circulating in the maternal plasma.

The methods used in all cases are mostly based on PCR amplification with allele-specific primers. **Results**: From our experience, the application of blood group genotyping methods, complementing the serological typing, has contributed to improve the quality and the accuracy of the typing results. Molecular analysis of blood group alleles has also allowed us to describe allelic variants, like the *D category VI type IV*, which is the most frequent *DVI* allele in the Spanish population and yet has not been reported elsewhere. Likewise, alleles of african origin like the GATA-mutated *FY* allele or the hybrid *RHD-CE-*D^S ($r^{'s}$ haplotype), which are very rare in caucasian populations of Central Europe, are detected with a significant frequency in the Spanish population. These observations are in agreement with the geographical proximity of Spain to the North of Africa and their historical relationship. In any case, this valuable information has influenced in the design of the genotyping strategies we apply and it is taken into account in the interpretation of the results. **Conclusions:** Blood group genotyping is a helpful tool with a gradually increasing number of applications. Genotyping studies are allowing us to have a better knowledge of the blood group distribution and molecular bases in our population. This information will ultimately help us to improve blood group typing in our laboratories and blood banks.

SY107

Prenatal *RHD* testing of fetus and mother: Decision to administer anti-D prophylaxis

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Background: D-negative women receive antenatal anti-D prophylaxis irrespective of the RhD-status of the fetus. In about 40% of these women this gift is given unnecessary, because the fetus is D-negative. To identify women carrying D-positive fetuses, we have developed a fully automated assay for fetal *RHD* genotyping using cell-free fetal DNA from maternal plasma. **Methods:** 1 ml of maternal plasma is automatically presented (Tecan) to a DNA isolation-robot (Roche). The DNA-eluate is tested (after automatically pipetting) in triplicate in a real-time quantitative *RHD* exon 7-PCR (ApplBios). From Oct-Dec 2003, plasma from 2,397 (serologically confirmed) D-negative pregnant women, whose blood was sent in for 28th - 30th week antibody screening, was tested. The women were sent questionnaires on cord blood serology. **Results:** 1,470 of the plasma were typed RhD positive and 932 RhD negative. Positive and negative results could clearly be discriminated. Because the *RHD* exon 7-PCR will give positive results in D-negative women carrying D-negative variant *RHD* genes such as *RHDΨ,* we determined the incidence of these genes by analyzing DNA isolated from buffy coats. The frequency of these genes was 0.5 %. At present, of 1,257 newborns the serology is known. In 1,245 cases (99.1%) serology and PCR were concordant. In 7 cases the genotype suggests D-positivity while serology is D-negative. In 5 cases no *RHD*-sequences were detected in plasma but cord blood was typed D-positive. For the discrepant cases it cannot be concluded yet, whether serology or PCR is correct. The negative serological results cannot serve as golden standard, since we showed that 1.44% (35/2432) of the supposed D-negative pregnant women whose blood was sent in for antibody screening were serologically D-positive. The true *RHD*-state of these cases will be investigated by DNA tests on buccal swabs of the newborns. **Conclusions:** This is the first large-scale study demonstrating the feasibility of screening D-negative women to restrict antenatal anti-D to women carrying D-positive fetuses. When antenatal prophylaxis would have been given based on PCR results, then 39.8% antenatal gifts would have been saved. Furthermore, postnatal prophylaxis could have been given directly after delivery in all women with a positive PCR-result, saving cord blood serology tests and possibly increasing the effectiveness of postnatal prophylaxis. Non-invasive fetal *RHD*-genotyping is feasible in all D-negative pregnant women.

SY108

The industry perspective: Lessons from previous technology changes

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In the past 25 years routine diagnosis of blood groups has undergone several technology changes. The shift from polyclonal to monoclonal reagents, which started in the early 80ties, led to a high level of standardization. The introduction of new test systems like microtiter plate and gelcard not only had an impact on the quality of manual testing, but was also a basic requirement for automation in all fields of application. Although automation is promoted by factors like cost pressure, increasing non-availability of qualified personal, legislation and regulatory requirements, the actual level is still low in mid sized and small laboratories. Besides user requirements, experiences from previous technology changes in blood grouping as well as in other diagnostic areas have to be considered during design phase, when a totally new technical platform like genotyping is introduced in routine blood grouping. In HLA-diagnosis a technology shift from serology to molecular biology was initiated more than 15 years ago. This technology change was driven by the demand on improved typing quality, primarily in HLA Class II. Today three different technologies, sequencing SSP and SSO, are available. In SSO not less than 3 different assay formats are used in routine diagnosis. But, although molecular typing led to improved diagnostic quality with much higher information level, the average price for molecular typing is decreasing. On the other hand, molecular assays are much more complex compared to serological assays, a fact that results in higher production costs. For these reasons all activities to introduce micro-arrays in molecular typing have not been successful so far. At present routine bloodgrouping demands technical expertise but is safe and test prices are affordable. This and a high level of regulation represent high entrance barriers for new technologies. In conclusion, the introduction of genotyping test systems should focus in first phase on special applications like blood grouping in polytransfused individuals or Rhesus prophylaxis, in which classical serological techniques often fail to deliver adequate results. The introduction of mass genotyping for blood groups, however, can only be successful when market and user requirements, regulatory issues as well as experiences from previous technology changes are considered.

Friday, September 24, 2004: Supplementary Training

FB3: Accreditation and Certification in Transfusion Medicine

FB301

Zertifizierung und Akkreditierung von medizinischen Laboratorien nach ISO 9001 und ISO 15189

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In den letzten Jahren hat das Thema Qualität in allen Bereichen der Gesellschaft stark an Bedeutung gewonnen. In medizinischen Einrichtungen wurde und wird dem Thema Qualität mit der Einführung von Qualitätsmanagementsystemen Rechnung getragen. Viele Krankenhäuser haben beispielsweise Qualitätsmanagementsysteme nach der DIN EN ISO 9001:2000 eingeführt und die Umsetzung und Wirksamkeit sich im Rahmen einer Zertifizierung bestätigen lassen. Wesentlicher Inhalt eines Qualitätsmanagementsystems nach ISO 9001:2000 ist die Beschreibung von Prozessen, die Kundenorientierung und die ständige Verbesserung. In Laboratorien hat die Qualität eine ganz besondere Bedeutung. Hier kommt es nicht nur auf die Beschreibung und Sicherstellung von Prozessen an, sondern darüber hinaus in besonderem Maße auf die Kompetenz der Laboratorien, bestimmte Untersuchungen richtig durchführen zu können. Dieses zusätzliche Qualitätsmerkmal ist wesentlicher Bestandteil der internationalen Norm DIN EN ISO 15189 „Medizinische Laboratorien - Besondere Anforderungen an die Qualität und Kompetenz“. Die ISO 15189 beinhaltet entsprechend nicht nur Anforderungen zum Qualitsätsmanagement gemäß der ISO 9001:2000, sondern zusätzliche fachliche Anforderungen. Dazu gehören u.a. konkrete Anforderungen an das Personal, die Räumlichkeiten, die Präanalytik, die Untersuchungsverfahren und deren Validierung, die interne und externe Qualitätskontrolle und die Befunde (Berichte). Der Nachweis, dass ein medizinisches Laboratorium die Anforderungen der ISO 15189 erfüllt, wird durch eine Akkreditierung durch die DACH Deutsche Akkreditierungsstelle Chemie bestätigt.

FB302

Transfusion Medicine Requirements for an ISO and GMP conform Quality Management System

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Safety of blood and tissues is an important focus of national legislative regulation and has been addressed by the European union directive to provide an consistently high blood product standard throughout Europe. Based on regulations and guidelines quality safety and in particular quality management systems have gained considerable importance in controlling (A) blood procurement and processing and (B) economic aspects in modern

transfusion medicine. Product safety is furthermore an essential requisite in transfusion medicine to minimise detrimental side effects of hemotherapy and to improve therapeutic intervention by novel cellular techniques including stem cell biology.
These demands emphasis a tight link between blood transfusion services and hospitals in order to achieve best practice. While GMP is focused on processes, ISO 9000 lays down overall management structures. We have therefore established an extended quality management system for blood transfusion services combining certification of the quality management system according to ISO 9001 / 13485 and accreditation of diagnostic areas according to ISO 15189. This system uses the ISO structure in order to include current GMP/PIC standards as well as technical standards for diagnostic procedures. The quality management handbook and the supplementing general procedures describe blood component production, laboratory testing and autologous and homologous blood transfusion. Based on the ISO 9001 quality elements (QE) it fulfils high demands in the control of producing blood components and diagnostic results by risk assessment and validation of all processes involved. It is further suitable to optimise processes, reduce errors and increase costumer satisfaction leading to an improved cost-benefit relation and a high quality level for blood components and transfusion services provided to hospitals and patients.

FB303

Quality Management and Complaint Handling for Medical Devices

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Quality Management (QM) is the management of all relevant processes within a company on the basis of a complete description of the internal processes and subsequent permanent adaptation and optimisation. QM may therefore be addressed for each malfunction of a process within a company - but not for defect products. Product quality is produced and it is only produced in Production or in Research and Development (R&D). Production and respectively R&D are therefore responsible for defect - free products.
Regarding the term quality it has be differentiated between Process Quality and Product Quality which have their origin in the correct design of a Process and Product respectively.
Both product and process design needs the highest priority in a producing company as mistakes here will highly influence the risk with a marketed product.
By means of a working QM-System these defects may be prevented if processes are carefully described and lacks of needed information can be localised early.
But QM is not restricted to products and production processes.
All processes of a company like for example: Company Risk Management, Financial and Quality Scores, Budgeting, Public Relations, R&D Projects, Management Reporting, Customer Satisfaction, Product Change, Complaint Process, etc. needs to be reviewed by the QM-System.
One of the central processes - probably the most important process - is the availability of market information. A multinational company like Fresenius HemoCare needs to be able to access market information of both own - and competitor products. A worldwide linked information system of market reports and complaints is used to identify the relevant information as well as to inform internally the relevant management members and safety officers in order to be able to decide about safety relevant information within seconds - happening anywhere in the world.
The information system for complaints is internet based and forwards automatically any needed information to the responsible quality assurance organization of the production site which has produced the product.
The address where to send the sample is also automatically sent back to the sender. The answer follows after investigation of the root-cause. Corrective and preventive actions are defined for each complaint at the production site which may include R&D- or Sales- linked activities like redesign or customer trainings.
The quality status of each product is reported to the responsible product manager who is the person in charge to improve safety and quality of a product. The information follows a pre-defined work flow without the necessity that the sender knows whom and where to send the information.
Another important process with respect to design quality is the product change process. A product change may be asked by any employee or from customer site via the sales representative but it needs carefully being evaluated by responsible persons of the R&D department and the production site. A risk analysis according agreed standards needs to be performed.
Besides that all major project needs also to be evaluated and decided by the management team within the view of the agreed budget. Also that process can easily be harmonized by the usage of a work flow system.

FB4: Hemostaseology

FB401

Massive bleeding in trauma: The multi-factorial mechanism of coagulopathy and the role of rFVIIa in its treatment

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Uncontrolled hemorrhage is a major cause of death in trauma patients, accounts for about 40-50% of the mortality in both military and civilian trauma. Most critically ill trauma patients develop profound multi factorial coagulopathy due to: DIC-like coagulopathy due to activation of coagulation with consumption of coagulation factors and platelets, hyperfibrinolysis, hemodilution; acidosis, massive transfusions and hypothermia. Introduction of a "site-specific" agent enhancing hemostasis at the site of injury may decrease hemorrhagic mortality and morbidity in trauma patients. Such agent - rFVIIa - has been used successfully for almost a decade in patients with hemophilia developing inhibitors to factor VIII or IX. rFVIIa activates the coagulation system on the membrane of tissue factor bearing cells and activated platelets at the site of injury. Hence, its action is compartmentalized, limited to the site of injury, without a systemic effect. The use of rFVIIa in trauma patients was avoided due to the theoretical risk of thromboembolic complications. We performed a pig trauma study that supported the safety and efficacy of rFVIIa in this animal trauma model and immediately after few exsanguinating trauma patients were treated successfully with tFVIIa, which led to ethical committee approval of this agent in patients suffering uncontrolled bleeding. **Patients:** Since mid 1999 over 250 trauma, surgical and medical patients suffering massive, life-threatening bleeding, have been treated with rFVIIa in Israel. We describe here the data of the first 36 trauma patients treated in 14 out of the 22 hospitals in Israel. Patients were critically ill, (ISS 25-75 in 86%) multi-transfused (see below) hypothermic, acidotic and suffered profound coagulopathy (see below). Median age was 20 (range 14-65), most were victims of terror (40%) and other violence (32%), which reflected on the type of injury (46% penetrating, 11% blast and the rest blunt trauma). **Results:** The abnormal coagulation tests improved significantly within 15-20 min. after administration of rFVIIa [PT shortened from 20.7±8.4 to 13.3±6.3 sec. $p<0.005$ and aPTT from 75±41 to 55.7±31.9 sec $p<0.005$). Cessation of bleeding was observed in 27/36 (75%) of the patients after administration of 1-4 doses of rFVIIa. Blood requirements decreased dramatically from 29.2±22.2 units of red packed cells given within 5.2± 4 hours to 5.2±6.19 units given over the next 24 hours (2,2±2.5 in the survivors) $p<0.05$. Twenty two patients (61%) survived. Most common cause of death was exsanguinations [8/14 (57%)] followed by sepsis [4/14 (28.5%)] and SIRS (2/14). Acidosis was found to be an independent risk factor for no response and mortality. There is also an impression that rFVIIa is less effective below pH of 7.1 but the number of patients is too small to conclude. A trend for a better survival was found in patients who received higher first dose [median 120/ 98-138 (25-75 IQR) µg/kg] vs. those who received 96/ 50-120(25-75 IQR) µg/kg but larger numbers are required to evaluate this point. Similar results were obtained by other groups (will be presented in the lecture).
Suggestions: rFVIIa seems to be a promising adjunct hemostatic agent in trauma patients suffering massive hemorrhage..
Future perspectives: Recent evidence from clinical cases suggest that rFVIIa may have beneficial effect in traumatic brain injuries and blast lung injures and may also improve and prolong survival upon pre hospital administration (prolongation of the "golden hour"). Such preliminary evidence will be presented.

FB403

„Off-label Use“ von rekombinantem aktivierten Protein C

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Drotrecogin alfa (aktiviert) (DAA) ist ein rekombinantes humanes aktiviertes Protein C, das für die adjuvante Therapie der schweren Sepsis zugelassen ist. In klinischen Studien zeigte sich eine Verbesserung derÜberlebensrate bei Patienten mit schwerer Sepsis und Organdysfunktion unabhängig vom Vorhandensein einer disseminierten intravasalen Gerinnung. Die Therapie ist allerdings bei Patienten mit niedriger Thrombocytenzahl als Indikator einer Organdysfunktion des vaskulären Systems, besonders effektiv hinsichtlich der Überlebensrate, so daß insbesondere auch Patienten mit Verbrauchskoagulopathie von einer Behandlung mit DAA profitieren.
In Fallberichten dokumentiert ist der Einsatz von DAA bei Patienten mit Sepsis-induzierter Purpura fulminans, wobei die Indikationsstellung Sepsis durch die Zulassung des Medikamentes abgedeckt ist. DAA ist hier eine Alternative zur ansonsten empfohlenen Protein C-Substitution.
Der Einsatz von DAA bei nicht-septisch induzierter Verbrauchskoagulopathie ist bisher nicht berichtet worden. Ein Einsatz von DAA bei Ischämie-Reperfusionsschäden wird diskutiert, ist bisher jedoch ebenfalls nicht durch Studienergebnisse am Menschen belegt.
Weiteres mögliches Einsatzgebiet von DAA ist die Therapie der nicht-septischen Purpura fulminans bei schwerem angeborenem Protein C-Mangel, wobei hierzu bisher keine Fallberichte oder Studien vorliegen. Denkbar ist außerdem ein Einsatz bei Patienten mit Thrombosen oder Embolien und Protein C-Mangel.
Vorteil von DAA gegenüber einer Substitution mit Protein C ist die Tatsache, daß eine endogene Aktivierung durch Thrombin/Thrombomodulin nicht erforderlich ist. Eine gestörte Endothelfunktion oder Mikrozirkulation hat daher ebenso keinen Einfluß auf die Wirksamkeit wie eine parallel durchgeführte gerinnungshemmende Therapie. Zusätzliche Varianten von DAA mit verminderter Bindung an endogene Inhibitoren oder verändertem Wirkprofil sind in der Entwicklung.

FB5: ADV – Automation and Data Processing

FB501

Computer assisted logistic in transfusion medicine

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The safe collection, production, distribution and application of blood and blood products in a high quality needs logistic on a high level. New developments in the eighties such as electronic data processing (EDP) and barcodes were implemented shortly after routine use in the industry. The nineties were the era of infection diseases. In connection with the development of new test a lot of data have to be managed and checked. New blood bank software and computers with enormous capacity moved into the blood establishments.
Another consequence of the scandals in the nineties was a series of legal regulations by the authorities in countries of the European Union, and Germany in particular. For e.g. blood banks have to work according to Good Manufacturing Practice (GMP) with documentation of every step of collection and production. Another important requirement is the possibility of look back and the implementation of a haemovigilance system.
On the other hand the SHOT-study shows, that the most frequent hazard of transfusions are the mistakes/ mismatches.
For all these problems existing various single point solutions such as patient-wristband, bed-side test, double check of blood group typing and donor - donation registry in software, etc.
The lecture will deal with new developments in logistics and data management, which can help to reduce the problems associated with the documentation, reporting and identification.

FB502

Automatic sample sorting improves blood safety

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Background: About 9000 donor samples are tested daily in our blood donation center. Samples are collected in tubes with different diameters (13 mm and 15 mm) and sorted in different instrument racks (Tecan, Olympus and Prism). Aim of our present study was to improve safety of our testing-procedure by implementing an automatic sample sorter (Sarstedt®). **Materials and Methods:** Unsorted samples were centrifuged in special centrifugation inserts which can be placed on the supply stage of the automatic sample sorter. The sample sorter red the sample-barcodes and compared the numbers with a working list. Subsequently samples were decapped and sorted into different instrument racks. After pooling with Tecan pipetting robot the samples were sorted again from Tecan racks into archive racks. Specific software of the automatic sample sorter identifies the samples during the whole process by barcodes. **Results:** 6000 samples from different customers were barcode scanned, decapped and sorted in different instrument racks (2000 in Tecan racks, 2000 in Olympus racks and 2000 in Prism racks). Subsequently 2000 samples from Tecan racks were sorted into archive racks. **Conclusion:** Sample sorter improves safety because the whole process is barcode-controlled, missing samples are recognized immediately. Tested samples could easily located in archive racks, which were also barcode labeled, for a renewed testing. Additionally the sample sorter is cost-effective. We saved time and personnel, because there is no longer a need for manual decapping of samples and sorting of samples in the different instruments racks.

FB503

NADIS-TMCP: LIMS for the management of pool-NAT testing of blood donations for HCV, HIV, HBV, HAV and Parvo B 19

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Purpose: The management of pool-NAT testing requires support by a LIMS that explodes the pool data to the individual donation and manages the formation of secondary pools for the identification of a positive donation in case of positive results. We extended our LIMS (NADIS) to automatically create worklists for the ABI Prism 7000 and 7700 sequence detection systems and to import the PCR results for further processing. **Methods and Results:** Pool information provided by pipetting machines and containing barcode numbers of the individual pooled donations is transferred to NADIS which allocates the samples to the individual customers. NADIS creates worklists for each virus and each team of technicians assigning the pool numbers and the controls to defined positions on the reaction plate. After pipetting the reagents and viral nucleic acids extracts into the reaction plate according to the worklist, the barcode number of the plate is scanned and linked to the worklist. At each real-time cycler, the plate barcode is scanned again and the virus-specific worklist is imported into the cycler software. After cycling a newly developed TaqMan control program (TMCP) performs computerized analysis of the PCR data (RN and CT) on the basis of predefined cut-off values to create a qualitative PCR result that is imported into NADIS. TMCP displays the amplification plot for amplified viral sequences and internal controls for the whole reaction plate as well as multicomponent views for each individual pool sample on one screen. Technician and physician can accept or overrule the results proposed by TMCP before transfer of the results to the mainframe computer for release of the blood products. To evaluate the benefit of our new LIMS, analysis of result transmission time was performed. Time of transmission of results to one of our customers in January was compared to time of result transmission in march. By the help of our new LIMS, results could be transmitted on average (median) 54 minutes earlier than before. **Conclusion:** By the use of modern IT, high throughput pool-PCR testing can be performed in a safe and efficient way.

FB504

Automated DNA Extraction by the Qiagen Biorobot MDX and import of storage & quality data into a LIMS – validation and analysis of 3390 samples

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Purpose: HLA-typing for our registry of volunteer stem cell donors requires efficient DNA extraction for up to 2000 samples per campaign. Through long-term storage of gained DNA, further typing of potential donors can be performed very fast. **Methods / Results:** We evaluated yield and quality of DNA extracted by the Biorobot MDX by analysing concentration and ratio of 96 EDTA whole blood samples, 32 fresh buffy coats and 32 buffy coats frozen at –30C. All samples were obtained from volunteer donors. The average DNA concentration for (A) fresh EDTA whole blood samples was 22.2 µg/ml (SD 6.9 µg/ml) with an average ratio of 1.94 (SD 0.05), (B) for fresh buffy coat (n=32) the average DNA conc. was 37.7 µg/ml (SD 28.7) with an average ratio of 1.72 (SD 0.18) and for (C) the 32 frozen buffy coats that have been stored at –30 °C for more than one year, the average DNA conc. was 23.6 µg/ml (SD 10.96), with an average ratio of 1.77 (SD 0.08). The extraction of stored buffy coats showed best results when these buffy coats were diluted with PBS. In total, we observed only in 3/160 (2%) samples DNA conc. of less than 10 µg/ml. This was the lower limit for reliable typing results. The automated DNA extraction resulted in a 100% specificity when compared to samples of known phenotype. The Biorobot MDX allows the integration of extraction data in a common LIMS (SwissLab) by a unidirectional interface that transfers sample number, number of and position on the storage plate and extraction status (valid/invalid). Using this interface, we analysed 3390 samples (394 buffy coat stored at –30° C for more than 1 year, 2996 whole blood samples stored for max. 14 days at 4°C). We observed 75 (2.2%) samples which were marked as invalid and had a DNA concentration below 10 µg/ml. The percentage of invalid DNA extractions with an concentration below 10 µg/ml was much higher in buffy coats (8,4 %; n=33/394) compared to whole blood (1,4%, n=42/2996). Discrepancy in extraction efficiency between whole blood and buffy coat was mostly due to low volume or clotting of stored buffy coat samples. **Conclusion:** automated DNA extraction with the Biorobot MDX allows efficient DNA extraction from volunteer stem cell donors and import of storage and quality data of the DNA directly into a LIMS. The technology allows for high-throughput DNA extraction of 288 (-384) samples per day by one technician.

FB505

Computer assisted management of a hospital blood inventory

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Purpose: The management of a hospital blood inventory primarily has to ensure the maintenance of blood supply, even in emergencies. However, transfused red blood cells (RBC) should be as fresh as possible whereas outdating of blood has to be avoided on economical reasons. We tested whether a computer assisted blood inventory management system could integrate the latter demands. **Methods**: Data acquisition about blood inventory stock size, numbers and age of transfused RBCs and numbers of outdated RBCs were collected during a 4 year period. After the first two years an intervention was done. Amongst other measures, weekly computer print outs of actual stock lists of RBC at risk of outdating (< 10 days residual shelf life) were generated. RBCs at risk of outdating were identified and allocated to transfusions definitely taking place within the residual shelf life. **Results**: The annual turnover of RBCs and the average actual stock size were stable during the 4 year period. Whereas there were only marginal changes in the mean age of transfused RBCs (17.5 – 17.7 days), the rate of outdated RBCs significantly decreased from 0.44% before intervention to 0.15%. **Conclusions**: Computer-assisted management of a hospital blood inventory significantly reduced outdating of RBCs. However, the mean age of transfused RBCs was not influenced and seems to be predominantly dependent from stock size and age of the RBC when freshly added to the stock.

Poster

PO0101

Cryopreservation of hematopoietic stem and progenitor cells from umbilical cord blood

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[4] STEMMAT is a bavarian joint research project to investigate adult stem cells from umbilical cord blood and tissues and is supported by the Bavarian Government.

Purpose: Umbilical cord blood (UCB) is an important source for adult hematopoietic stem and progenitor cells (HSC, HPC). These HSC have been shown to be equivalent to bone marrow stem cells for reconstitution of the hematopoietic system. In addition they may be multipotent and capable of differentiating into other cell types. Cryopreservation of these cells is critical for UCB banking and transplantation. **Methods:** We have developed protocols for cryopreservation of UCB hematopoietic stem and progenitor cells after mononuclear cell preparation. Reproducible freezing conditions were established using a computer controlled freezing protocol.**Results and Conclusions:** After cryopreservation high viabilities (89%) and recoveries for $CD34^+$ cells (92%) were observed. In addition, using a colony formation assay, a high proportion of the $CD34^+$ cells were shown to be functional, leading to a recovery of 83% for total colony forming units (CFUs). The use of human albumin compared to autologous plasma in the cryomedium as well as freezing at high cell concentrations proved to be beneficial for the recovery of functional $CD34^+$ cells. A protocol involving slow, stepwise addition and removal of DMSO prior to and after cryopreservation in order to avoid osmotic stress for the cryopreserved cells was compared to our standard protocols. Our results show that fast addition and removal of DMSO yields higher recoveries of $CD34^+$ cells and total CFU (89% compared to 77% and 89% to 73%, respectively). Interestingly, preliminary data suggest that a combination of cryopreservation protocols including fast addition of DMSO before freezing and slow dilution after thawing seemed to be at least as efficient (98% recovery for $CD34^+$ and 86% for total CFU) as the previously described protocol with fast addition and removal (89% and 89%, respectively). Thus we have developed an efficient protocol for cryopreservation of HSC and HPC from UCB.

PO0201

Progenitor cells mobilized after myocardial infarction related to patient age

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With the intention to support regeneration of cardiac tissue after myocardial ischemia (mi) autologous progenitor cells have recently been used. In a series of 13 patients we performed flow cytometric determination of progenitor cells. At the time of invasive diagnostics and seven days later in patients suffering from myocardial infarction beside routine laboratory parameters CD34 positive cells were determined by flow cytometry. High resolution magnetic resonance image analysis was used to describe cardiac damage. Comparing results generated at the time of mi to measurements performed seven days later increase of relative amount of CD34 positive cells among total nucleated cells was demonstrated. In this small group of patients being 37 to 79 years of age increasing concentration of progenitor cells over time was inversely related to patient age. Mobilisation kinetics of CD34 positive cells in patients early after myocardial infarction indicated possible endogenous repair efforts. These results should be of interest to plan therapeutic strategies intended for improvement of usually insufficient recovery from myocardial infarction.

PO0202

Stem Cells for the Replacement of Retinal Pigment Epithelium in Age Related Maculopathy

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Purpose: Age related maculopathy (ARM) is a chronic, progressive disease of the macula that results in the loss of central vision. The non-exudative ("dry") form of ARM, representing approximately 90% of all patients, is characterized by a degeneration of the retinal pigment epithelium (RPE). Replacement of degenerated RPE cells by transplantation of adult stem cells appears as a feasible causative therapy. Our project aims at localizing, isolating and cultivating remnant stem cells in the adult eye and analyzing the differentiation capacity of these cells. **Methods:** The broad ciliary marginal zone (CMZ) of rat eyes was excised and consecutively digested. Subsequently a suspension cell culture was established using optimized medium for primary neuronal cells. To ensure optimal growth conditions of spheric cell aggregates, 20ng/ml basic fibroblast growth factor (bFGF) and 10 ng/ml epidermal growth factor (EGF) were added. Differentiation of the cells was induced by 1-10% fetal calf serum (FCS) after adherence of the spheres to a Polyethyleneimine coated surface. The differentiation pattern was assessed by immunohistological staining for neurogenic or RPE markers. Additionally the mitotic capacity was immuncytologically detected via BrdU incorporation. **Results:** Cell culture and differentiation conditions for primary cells derived from the broad CMZ of rat eyes at different ages could be established. A relativly high longevity of the cultured spheric aggregates was achieved with cells that have been cultured over a period of at least six months. Differentiated cells expressed nestin, indicating the presence of progenitor cells. Also, both glial (GFAP) and neuronal (MAP2) markers were expressed on cells migrating from the spheres. Finally, cells after differentiation were stained positive with the RPE markers cytokeratine 8 and 18 and bestrophin. The proliferation of differentiated cells was shown by BrdU labelling. **Conclusion:** Residual pluripotent cells can be isolated from the ciliary marginal zone of the adult rodent eye. These cells can be cultivated over a period of several months and can be induced to differentiate into cells expressing neuronal, glial and RPE markers. Further characterization of the cells shall evaluate whether a direct surgical transfer of cultivated specimen into the subfoveal space might be beneficial to replace RPE cells in non-exudative age-related macular degeneration.

PO0301

Intra-Individual Comparison of Donor Lymphocyte and Blood Stem Cell Apheresis

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Purpose: Donor lymphocyte (DL) infusion as adoptive immunotherapy in allogeneic stem cell transplantation has gained considerable interest. Due to conflicting results on the application of lymphocytes from unstimulated and cytokine-stimulated donors we compared the yields of particular cell types including lymphocyte subpopulations in the apheresis products. Because the effect of rhG-CSF can vary considerably an intra-individual comparison was performed. **Methods**: 57 healthy related allogeneic stem cell donors (38 males, 9 females) with a median age of 45 years (range 16-73) performed a continuous-flow apheresis using a COBE Spectra (Gambro) or a COMTEC (Fresenius) cell separator. The apheresis product was analysed on a blood counter (KX-21, Sysmex) and a flow cytometer for differential count and the determination of CD34+ cells and lymphocyte subpopulations (Calibur, BD). **Results**: DL apheresis with the unstimulated donor lasted shorter (175 vs. 250 min) with less blood volume processed (11.4 vs. 18.9 L) than blood stem cell (PBSC) apheresis. In total 125 procedures, i.e. 1.0 DL and 1.2 PBSC aphereses per donor were performed. In 14 collections (8 in untreated,

6 in rhG-CSF stimulated donors) adverse events were recorded. Donor reactions were mild, hypotension being the most common. Due to inadequate inlet flow 2 procedures were terminated early. The median yields from untreated vs. rhG-CSF stimulated donors were: total nuclear cells (TNC) 1.5*10E10 vs. 12.0*10E10, 1.2*10E10 vs. 3.2*10E10 lymphocytes, 7.2 vs. 22.4 *10E9 T lymphocytes, 1.2 vs. 4.6*10E9 B lymphocytes, 1.4 vs. 2.2*10E9 NK cells, and 0.03 vs. 5.3*10E8 (n=5) respectively. PBSC harvests (n= 68) of each donor yielded 6.1*10E8 CD34+ cells or 8.9*10E6 CD 34+ cells/kg recipient. The extraction efficacy (EE), i.e. cell yield/donor body weight [kg]*volume of blood processed [L], for CD34+ cells was about 200fold higher with rhG-CSF stimulated than that with untreated donors. rhG-CSF application resulted in a moderate (1.5 to 4.4 fold) increase of EE for TNC, B and T cells, that for NK cells and platelets remaining unchanged**.** **Conclusion**: Needs for apheresis collection of effector cells in allogeneic hematopoietic transplantation can safely be met often in a single procedure. Stimulation of donors with rhG-CSF results in a highly specific increase of CD34+ cells. As compared to other lymphocyte subpopulations the decrease of NK cells in PBSC harvests may contribute to the shift towards a Th2 profile.

PO0302

Collection of MNCs with two different cell separators for adoptive immunotherapy

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Background: Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system and therefore increasingly used in adoptive tumor therapy. DCs can be generated from peripheral monocytes usually collected by mononuclear cell (MNC) apheresis. We investigated the quality of autologous MNCs collected with either the Amicus device (Baxter), or AS.TEC device (Fresenius) assessed by the differentiation of monocytes into DCs. **Material and Methods:** Fifty patients suffering from malignancies (renal cell cancer = 6, melanoma stage III/IV =34 and others = 10) underwent leukocyte apheresis either with the Amicus (n = 27, processed blood volume was 10.236 mL ± 895) or the AS.TEC (n = 23, processed blood volume was 9953 mL ± 1522 .). MNCs of the products were isolated by density gradient centrifugation, subsequently washed and cultured according to standard protocols for 6 days. Thereafter DCs were pulsed with tumor antigen and cultured for another 3 days in medium containing proinflammatory cytokines which induces DC maturation. Mature DCs were characterized by FACS analysis. All blood cell and differential counts were performed with the Sysmex F-820 device (Kobe). For statistical analysis the Mann-Whitney U test was applied. **Results:** No differences were found in the monocyte content of either apheresis product (p=0.07) and the overall yield of MNCs (p = 0.4). The total amount of cells harvested at day 6 of culture was 567 x 10^6 ± 291 for Amicus vs 517 x 10^6 ± 323 for AS.TEC, respectively, (p = 0.5). Also the final yield of mature DCs (evaluable of 25 Amicus collections and 11 AS.TEC collections) revealed no significant differences: AMICUS: 138 x 10^6 cells vs AS.TEC 185 x 10^6 cells (p = 0.5). The yield of mature DCs in relation to the number of originally harvested monocytes was 7,2% in case of Amicus and 8.4% when the AS.TEC device was used. **Conclusion:** Amicus and AS.TEC are equivalent in providing mononuclear cells for the generation of DCs.

PO0303

Exposure to Mercury after Immunoadsorption Treatment is Correlated with the age of Protein a-Sepharose Gel Columns

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Background: Immunoadsorption treatment with reusable protein A-Sepharose gel columns is increasingly used in therapy of IgG antibodiy mediated-autoimmune diseases. To keep the columns in excellent conditions and to prevent bacterial contamination during storage, they are preserved with mercury thiosalicylate (thiomersal 0.1%) after each procedure, which has to be removed again before treatment. Usually post-treatment values in patients do not exceed 10µg/L. In 1 patient an unexpected high mercury value was detected after treatment (132µg/L). This led to an attempt to determine parameters, which allow to predict mercury exposure during the treatment. **Patients and Methods:** In 6 patients a total of 63 treatments were performed (between 4 and 12 treatments for each patient). The columns were provided according to standard operating procedures. Mercury levels were measured by atomic absorption spectroscopy in whole blood and urine before and after each procedure as well as in the priming solutions and in the first 4 fractions of treated plasma. The column performance index (CPI), calculated by the microprocessor-controlled Citem 10 monitor reflects the condition of the column and thus provides information about the IgG adsorption capacity. Mercury values were correlated with the CPI using the Spearman rank correlations test. **Results:** A significant correlation was found between CPI and mercury levels in whole blood- and urine samples after immunoadsorption treatment and in the tested plasma fractions ($p < 0.005$). In new columns CPI ranges between 48 and 54. When CPI fell below 30, the mercury values in whole blood- and urine samples exceeded three times the post-treatment values compared with a CPI above 30 (16.7µg/L (5.7-33.2) vs 6.2 µg/L (2.4-17.4) for whole blood and 2.4µg/L (1.2-7.2) vs 0.75 µg/L (0.2-4.9) for urine samples, respectively. **Conclusion:** We observed that CPI is not only showing the adsorption capacity of the columns but also correlates with the mercury exposure of treated patients.

PO0401

Red cell Antibody screening and Blood grouping in a fast micro-plate agglutination method (FMAM)

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Background: Because of repeated centrifugation and washing steps most conventional blood grouping techniques such as direct or indirect hemagglutination or adherence methods are both time-consuming and labor intensive. This report describes a novel agglutination method for blood grouping, reverse typing with only one centrifugation step but without the need for washing. **Study design and Methods**: In a comparative study, 101 randomly selected blood samples from donors and 139 randomly selected patient's sera were tested for ABO grouping, D typing and antibody screening. Further 58 patient's specimens containing low titer alloreactive antibodies, 93 specimens containing high titer alloantibodies, all with known specificity and furthermore 6 specimens with weak D properties were tested in a blinded study. Two commercially available blood testing systems (Olympus and Microtyping system, DiaMed), served as reference methods.**RESULTS**: All 101 samples tested revealed similar results in both forward and reverse typing. Of 6 weak D samples tested 5 were clearly identified in both systems and one was found to be negative in the reference system using two different MoAbs of IgM origin. In the case of low titer alloreactive antibodies we calculated a sensitivity of 76% for FMAM and 35% for the reference method. Testing high titer alloantibodies the sensitivities were 95.5% and 86% respectively. The overall specificity for FMAM was 94.3% and 97.8% for reference system. **CONCLUSION**: For the screening of red cell alloantibodies the FMAM is more sensitive compared to the gel indirect antiglobulin test under the conditions used. The short incubation time, no washing steps and only one step centrifugation makes this method adaptable to a fully automated system.

Abbreviation: FMAM = fast micro-plate agglutination method

PO0402

Comparative clinical trial of the sensitivity of Solid Phase System Capture-R Ready-Screen versus Gel Centrifugation (DiaMed-ID Micro Typing System)

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Purpose: Different systems are in use for the detection of transfusion relevant red cell IgG antibodies. In addition to the classic tube test, the solid phase system and the gel card centrifugation test are available. The intention of the study was the comparison of sensitivity between Solid Phase Capture-R Ready-Screen system (Capture-R, immucor GmbH, Germany) and gel card Diamed-ID Micro Typing System (ID-System, DiaMed AG). **Methods**: 100 deep frozen patient sera with known antibody specificities were centrifuged two times (3.100 rpm, 11 min, Hettich Rotanta RP) after thawing (water bath, 37 °C). Following this, a dilution series in isotonic NaCl-solution was produced and tested parallel in Capture-R and ID-System. The test processing was carried out according to manufacturers' instructions and using their devices. The Capture-R was incubated for 30 min at 37 °C in Heraeus B15 incubator, followed by a washing step with CSW 100 by immucor and centrifugation at 3 min with 2.100 rpm (Heraeus Labofuge 400). The ID-System was incubated for 15 min at 37 °C (DiaMed Incubator 37S I), followed by 10 min centrifugation at 910 rpm (DiaMed Centrifuge 24S). Results were analyzed by manual reading followed by photo documentation. **Results**: 26 examinations could not be enclosed [missing reaction in both systems (n=15), double examinations (n=5), multiple antibodies (n=5), antigen not present at the panel (n=1)]. The table shows an summary of 74 analysed examinations:

Antibody	ID-System > 2 titer steps more sensitive	Same Titer/ 1 titer step difference	Capture-R > 2 titer steps more sensitive
D (n=18)		2	16
C, c, C^w, E (n=20)	1	2	17
Jk^a, Jk^b (n=6)			6
Fy^a, Fy^b (n=11)		9	2
K (n=11)		3	8
Lu^a (n=4)	4		
S, M, Le^a (n=4)			4
Total (n=74)	**5**	**16**	**53**

Conclusions: In 72% of the samples, a clearly higher sensitivity (> 2 titer steps) was shown at the Capture-R Ready-Screen as compared to the Dia-Med-ID Micro Typing System. Duffy antibodies are recognized approximately equally sensitive in both systems. The tested Lu^a antibodies could not be detected in the Capture, also 1 Anti-C^w and 1 Anti-E in the ID-System.

PO0403

ABO-Identity Test for Pretranfusion Bedside Testing in a Lateral Flow Device

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Purpose: Most fatal complications of blood transfusion are due to errors which occur at the patient bedside. Therefore, ABO-Identity testing at the bedside is mandatory in several countries (Germany, France, Austria). The purpose of this study was to develop a bedside test with few manipulations and simple interpretation of the results. **Methods:** A lateral flow device was designed with two separation membranes (two recipients or one recipient and one blood bag segment) equipped in a cassette housing each having an application zone and an detection area printed with parallel lines of antibody reagents directed against blood groups A and B. One drop of recipient whole blood or segment blood (in the experimental model EDTA-blood was used) is dropped in the recipient application zone, followed by the addition of two droplets of a low ionic washing buffer. Results can be read after 2 minutes. Positive results are recognized as dark red bands with a pink background, negative results are recognized by the absence of the respective band. In an alternate test format, positive results are shown by a plus (+) symbol and negative results by a minus (-) symbol. **Results:** From 40 EDTA-blood samples tested (10 each of blood group A, B, AB, O, including Ax, A3B), all blood types were identified correctly. **Conclusions:** The ABO identity test presented here has only two pipetting steps for recipient and segment blood each. Band-shaped stable end-point results facilitate interpretation. Both criteria may help to reduce the risk of bedside testing errors.

PO0404

Tube agglutination and gel microcolumn technique: A comparison of two methods for red blood cell antibody titration

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Purpose: Traditionally saline tube agglutination technique (tube method) is the approved method for antibody titration. Since 1990 the gel microcolumn technology (gel method) has gained widespread usage throughout the world as a routine method in red blood cell (RBC) serology. The purpose of our investigation was the comparison of titration endpoints obtained by gel method with those obtained by tube method. **Methods**: 65 serum samples of individuals with irregular RBC antibodies (8 Anti-C, 3 Anti-Cw, 26 Anti-D, 9 Anti-E, 3 Anti-Fya, 12 Anti-K, 2 Anti-Jka, 2 Anti-M) identified in routine antibody screening by indirect antiglobulin test (IAT), were tested. Serial twofold dilutions (range 2-16.000) were done by one single person using the same test cells. For the tube method we prepared a 3% suspension of RBCs and performed the dilutions with NaCl according to the Technical Manual of the AABB. For the gel method we prepared a 0,8% RBC suspension and performed antibody titration in low ionic strength-solution diluent (ID-Diluent 2, DiaMed Switzerland) according to the manufacturers instructions. **Results** were expressed as the reciprocal of the highest dilution that caused a macroscopic agglutination. Results: 41/65 (63,1%) of the investigated antibodies had no or only a onefold difference in the titration endpoints between the tube and the gel method. In 18/65 (27,7%) samples the titration endpoint of the gel method was twofold higher than in the tube method. Only 6/65 (9,2%) samples had a titration difference of three ore more dilutional steps. The titration endpoints of these six specimens were between 1000 and 16.000 in the gel method. **Conclusion**: The gel microcolumn technology has shown in several studies to be more sensitive in the detection of red blood cell alloantibodies. With respect to our antibody titration study, only antibodies with a very high titer in the gel method show a marked difference compared to the tube method.

PO0405

Comparison of four tests for the quantification of fetomaternal hemorrhage

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Purpose: Adequate immunoprophylaxis in Rhesus D-negative mothers with massive fetomaternal hemorrhage requires a correct quantification of fetal red blood cells within the maternal circulation. We compared four assays based on the detection of either fetal hemoglobin or Rhesus D-positive cells. ***Methods:*** In a first series of experiments varying amounts (0.1, 0.2, 0.4, 1 and 5 %) of D-positive adult red blood cells were mixed with D-negative cells. Quantification was studies by flow cytometry using human monoclonal anti-D and by a commercial Rhesus D consumption assay. In a second series 0.1 to 5.0 % of cord red blood cells were mixed with adult red blood cells and quantified by flow cytometric determination of fetal hemoglobin (HbF) and by the Kleihauer/Betke-test. ***Results:*** The percentage of measured D-positive red blood cells did not significantly ($p = 0.25$) differ from the theoretical value using anti-D flow cytometry, while the consumption test in 37.5 % of the determinations produced higher values especially in the range from 0.1 to 0.2 % D-positive cells. Flow cytometric evaluation of HbF frequently determined false low percentages within the range of 0,4 to 5.0 % ($p \leq 0.01$). The Kleihauer-Betke test produced false high results beneath 1 % of fetal cells. ***Conclusions:*** Flow cytometric determination of D-positive red blood cells within D-negative blood proved to be the most reliable assay for the quantification of fetomaternal hemorrhage. The determination of HbF in addition to false low values has the disadvantage that HbF in small amounts also can be found in adults thus leading to misinterpretation of the data.