Euretina Lecture

Ophthalmologica

Ophthalmologica 2017;237:1–10
DOI: 10.1159/000455809

Received: December 29, 2016
Accepted: January 2, 2017
Published online: February 3, 2017

The Blood-Retinal Barrier in the Management of Retinal Disease: EURETINA Award Lecture

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Keywords
Blood-retinal barrier · Macular edema · Retinal edema

Abstract
Retinal diseases are the main causes of blindness in the Western world. Diabetic retinopathy and age-related macular degeneration continue to increase in prevalence and as main causes of vision loss. Intravitreal anti-VEGF and steroid injections have raised new expectations for their successful treatment. These agents act by stabilizing the blood-retinal barrier (BRB). Our group defined the BRB by identifying for the first time the tight junctions that unite retinal endothelial cells and are the basis for the inner BRB, an observation later confirmed in retinal pigment epithelial cells and in brain vessels. A major role of active transport processes was also identified. Today, the BRB is understood to play a fundamental role in retinal function in both health and disease. Retinal edema, an ubiquitous manifestation of retinal disease, is directly associated with breakdown of the BRB and with vision loss. In its most common form (i.e., vasogenic edema), due to breakdown of the BRB, Starling's law of capillary filtration may be used to interpret the mechanisms of fluid accumulation in the retina. The main factors involved in the development of retinal edema are BRB permeability, capillary hydrostatic pressure, tissue hydrostatic pressure, tissue osmotic pressure, and plasma osmotic pressure. In the clinical environment, breakdown of the BRB has been identified by fluorescein angiography and vitreous fluorometry, requiring the intravenous administration of fluorescein. An OCT-based method, OCT-Leakage, recently introduced by our group is capable of noninvasively identifying and quantifying sites of alteration of the BRB by mapping areas of lower-than-normal optical reflectivity, thus reflecting changes in the retinal extracellular fluid. We found good correspondence between the location of increased areas of low optical reflectivity identified by OCT-Leakage and the main sites of leakage on fluorescein angiography. Furthermore, with OCT-Leakage the areas of abnormal fluid accumulation can be identified in specific retinal layers, clearly offering more information than previously obtained with fluorescein angiography. OCT angiography has become available, replacing much of the information yielded by fluorescein angiography in a noninvasive manner. However, OCT angiography cannot visualize the leakage, i.e., the alteration of the BRB. OCT-Leakage is able to identify the locations of increases in extracellular fluid in the different layers of the retina. The complementarity of these 2 methods is of potential great interest for the diagnosis and management of several retinal diseases in which the presence and amount of fluid, as a marker of severity and activity, is paramount to treatment and management decisions in clinical practice.

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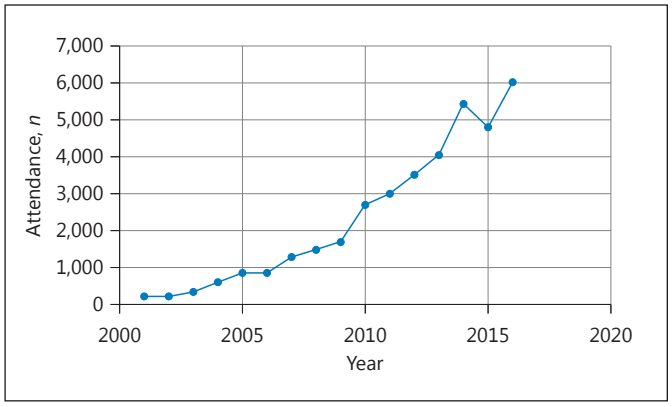

Fig. 1. EURETINA: its growth and consolidation as an international retina society.

It is a great feeling for me to be here, today, giving the EURETINA Award Lecture, the main invited lecture of my scientific society. The retina has been the object of my continued interest and scientific curiosity for many years, that is, since I finished my medical school years. I was trained in Coimbra and London and worked for 2 very successful periods in Chicago, USA. Finally, it was in Portugal, in Europe, that I decided to work and pursue my research activities. I also feel that I am truly honored to be in the company of the previous EURETINA Lecture awardees, many of whom are my personal friends. I was involved in the development of the European Society of Retina Specialists (EURETINA) and I am extremely happy to register its continued growth. It has now approximately 6,000 registrations. It currently is the main global scientific retina society, and its relevance has been growing steadily with participants coming from all over the world (Fig. 1).

It is between friends and colleagues with the same interest in the retina that I chose to share my personal perspectives on the relevance of the blood-retinal barrier (BRB) in retinal disease management. Everyone coming to the EURETINA Annual Meetings is looking for a better understanding of retinal eye diseases in order to treat their patients better and offer a more learned and intelligent approach to their disease.

Retinal diseases are the main causes of blindness in the Western world. Diabetic retinopathy is the leading cause of blindness in the working-age population. A meta-analysis of 35 studies (1980–2008) looking at the global prevalence of diabetic retinopathy showed values of 34.6% for any form of diabetic retinal disease and of 10.2% for vision-threatening diabetic retinopathy, i.e., macular edema and proliferative retinopathy. It is really a major health problem to realize that there are approximately 28 million people with vision-threatening diabetic retinopathy worldwide [1].

Age-related macular degeneration is the leading cause of blindness among individuals older than 50 years. The continued increase in lifetime years and longevity contributes to a continued increase in the prevalence of age-related macular degeneration and its predominance as a cause of vision loss. Recent progress in the treatment of retinal disease using intravitreal administration of anti-VEGF drugs or steroids has, however, completely changed expectations regarding successful vision recovery. The translational work that established intravitreal anti-VEGF therapy for retinal diseases was recently recognized as a landmark in the treatment of vision loss and received the 2014 Champalimaud Award [2].

It is important to realize that intravitreal anti-VEGF and steroid injections act by stabilizing the BRB and correct abnormal permeability in retinal disease. The concept of the blood-brain barrier (BBB), which first appeared in the literature in 1885, had its origin in the classic experiments by Goldman, who used trypan blue for the first time to demonstrate that at the blood-brain interface there is a barrier system that protects the brain.

In 1965 we published our study on the effect of histamine on the permeability of the ocular vessels [3]. Histamine markedly increased the vascular permeability of the various ocular tissues except for the retina. This behavior of the retinal vessels was similar only to what had previously been described for the cerebral vessels [4–6]. Following on our morphological studies and permeability measurements [4, 7], I proposed that the BRB should be regarded as consisting of 2 major components, the endothelium of the retinal blood vessels (inner BRB) and the retinal pigment epithelium (RPE) (outer BRB) [8].

Morphological studies, using electron microscopy, where extremely rewarding, demonstrating the presence of zonulae occludentes in the retinal vessels between the endothelial cells, thereby showing that the retinal endothelial layer has an epithelium-like structure and offering an explanation for the permeability behavior of the retinal vessels [6]. This observation was later confirmed also for the brain vessels, providing support for our present understanding of the important role of the endothelial layers of the retinal and brain vessels in the BRB and BBB, respectively [9]. Our studies also showed for the first time that there was an active transport of an organic anion (fluorescein) out of the vitreous body across the pigment epithelium and across the blood vessels of the retina [4, 7].

The term "BRB system" is most useful for clinical purposes and better signifies its major role, that is, regulating the microenvironment of the retina. The BRB system must be viewed as whole, and as regulating both the extracellular fluid of the retina and the vitreous. Today the BRB is understood to play a fundamental role in retinal function in both health and disease. The major diseases that affect visual function, i.e., diabetic macular edema and "wet" age-related macular degeneration, are characterized by breakdown of the inner and outer BRB, respectively. Macular edema is identified by swelling of the central portion of the human retina and is associated with increased retinal thickness. It can be simply defined as excess of fluid within the retinal tissue [10].

In order to understand the mechanisms of retinal edema it is necessary to consider data from many sources including anatomy, physiology, pathology, and clinical ophthalmology, as well as information obtained when dealing with the parallel situation in the brain. Like in the brain, free leakage of fluid and protein from the macular vasculature is prevented by the BRB. Thus, the interstitial spaces of the retina are normally relatively dry. Slow percolation of water into the retina from intraocular pressure may occur, but without solutes it is not retained, and active transport across the RPE removes the water as fast as it oozes through [11].

It must be realized that the normal retina possesses a functional extracellular space. Smelser et al. [12] injected Thorotrast particles into the vitreous humor of cats and were able to detect its presence within 6 h throughout the retinal extracellular space between the internal and external limiting membranes (ILM and ELM). They concluded that the intercellular space in the retina is available for diffusion of even particulate matter and that such clefts are important for retinal nutrition and could potentially play a role in pathological processes.

With regard to the extracellular volume of the retina there has been little physiologic investigation, although numerous studies have been conducted on the brain tissue of various species. In a major review of the subject, Cheek and Holt [13] concluded that the distribution of chloride offered the most accurate assessment of the brain's extracellular space. They reported values of 24.8% in the cerebrum and 23.6% in the cerebellum of adult rhesus monkeys. It is, therefore, accepted that the retinal extracellular space is of a similar size, functions as a system of interconnected pathways of varying dimension, and is present throughout the retinal tissue.

It is generally agreed that the most frequent proximate cause of macular edema, associated with any systemic or ocular disease or drug, is breakdown of the inner BRB. Although the contents of the vascular lumina can reach the extravascular space by transcellular mechanisms directly through the endothelial cell cytoplasm, most of the available data indicate that the most frequent pathologic mechanism is breakdown of the interendothelial junctional complexes [7]. The accumulation of fluid in edemas may be extracellular, intracellular, or a combination of both. In the retina, we may follow the classification proposed by Klatzo for brain edema [14].

Cytotoxic edemas are those in which the primary lesion is initiated in cells, neurons, or glia. Intracellular swelling of the retina may occur primarily with certain kinds of intoxication and in the margins of hypoxic/ischemic damage, where breakdown of the BRB is a secondary event [15]. Vasogenic edemas, which are by far the most frequent, are those in which the primary defect is in the BRB, leading to abnormal accumulation of extracellular fluid. In this situation, a secondary cellular response may occur in an effort to deal with the excess fluid in the extracellular space. Müller cells and aquaporin 4 channels may indeed be involved in the reabsorption of abnormal fluid entering the retina due to alterations of the BRB.

When there is a breakdown of the BRB in vasogenic edema, it is possible to interpret retinal edema in terms of the basic principles of capillary filtration. In 1896 Starling originally suggested that edema occurs in a tissue when the rate of capillary filtration exceeds the rate of fluid removal from the perivascular interstitium [16]. Lymphatics remove fluid from peripheral tissues, but fluid from retinal capillaries must percolate through the retina to reach the vitreous or return into the circulation.

In 1984 we proposed how Starling's law could be applied to the retina [17]. The "force" driving water across the capillary wall is the result of a hydrostatic pressure difference (Δp) and an effective osmotic pressure difference ($\sigma\Delta\pi$). Thus, the equation is:

$$(\text{flow}) = Lp\,[p^{\text{plasma}} - p^{\text{tissue}} - \sigma(\pi^{\text{plasma}} - \pi^{\text{tissue}})], \quad (1)$$

where Lp is the hydraulic conductivity or "membrane permeability" and σ is an osmotic reflection coefficient.

Retinal capillaries are different from most other peripheral capillaries, being almost impermeable to proteins, electrolytes, and water-soluble nonelectrolytes. The capillaries are lined by continuous endothelial cells with tight junctions, across which each of these solutes exerts full, effective osmotic pressure. At a first approximation, the protein osmotic pressure equals zero in retinal tissue, because protein is negligible in the vitreous and retinal

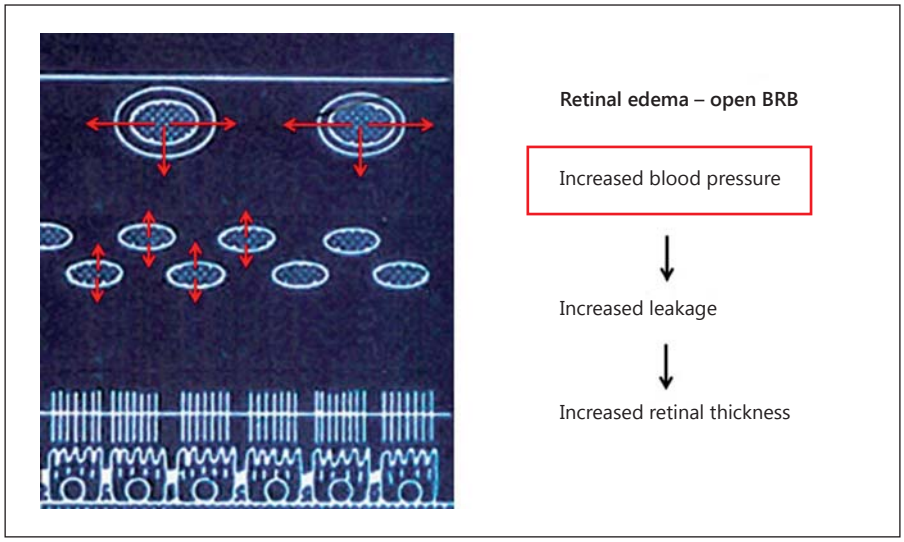

Fig. 2. Schematic drawing of the retina indicating how increased blood pressure may play a role in retinal edema when there is an alteration of the inner blood-retinal barrier (BRB).

extracellular space. Normally, then, capillary hydrostatic and protein osmotic pressures dominate the force term in the equation. π^{plasma} is 25 mm Hg higher than π^{tissue} because of protein osmotic pressure. p^{plasma} can be estimated by assuming that arteriolar resistance reduces carotid artery pressure from 65 mm Hg by about 50%. If p^{plasma} = 30 mm Hg, the driving force for filtration is <5 mm Hg, depending on the value of p^{tissue}.

Edema develops when fluid accumulates within the retina so as to increase the retinal volume. The rate of fluid accumulation depends on a fundamental factor, retinal compliance, and is more rapid in compliant tissue than in tissue that resists deformation when p^{tissue} rises.

The main factors influencing retinal edema formation therefore are the following:

(1) BRB permeability: An increase in BRB permeability is induced by direct damage to the retinal vessel wall and/or to the choroid-RPE complex. Another mechanism is loss of blood flow autoregulation. Increased permeability is reflected in Equation 1 as an increase in hydraulic conductivity and a fall in σ, both effects increasing BRB filtration.

(2) Capillary hydrostatic pressure: An increase in systemic blood pressure does not normally produce retinal edema, because blood flow autoregulation prevents capillary hypertension, dilatation, and breakdown of the barrier. However, edema often develops when autoregulation is overcome. Disease and loss of autoregulation lead to abnormal BRB permeability, allowing a full, direct effect of systemic blood pressure on fluid accumulation in the retina (Fig. 2).

(3) Tissue hydrostatic pressure: This term counterbalances capillary pressure and is altered in a situation of edema. In this situation there is more fluid between the cells, less tissue resistance, and consequently increased tissue compliance. Increased tissue compliance allows easier fluid accumulation in situations of increase in extracellular space (Fig. 3) and when there is vitreoretinal traction (Fig. 4). A normal, low retinal compliance in the closed eye could prevent measurable retinal edema in response to small increases in BRB filtration. The concept of retinal compliance may have particular importance and should be well understood. Low compliance of a tissue represents its resistance to deformation and accumulation of fluid within its limits. The retina has closely interwoven cellular processes, many junctions between glial cells, and a reduced extracellular space. Müller cells form a relatively rigid framework which holds together the remaining retinal tissue. It is this skeleton that must yield as a result of tissue damage or breakdown of the BRB, resulting in edema and thickening of the retina. In a situation of breakdown of the BRB, the accumulation of fluid in the retina increases its compliance, creating conditions for lower tissue resistance and further accumulation of fluid.

(4) Tissue osmotic pressure: Assuming that the amount of protein in the vitreous equals that in the retinal extracellular space, protein osmotic pressure in the retina is close to zero. A barrier opening allows proteins to enter the retina, contributing to edema formation. Tissue osmotic pressure may also increase as a result of an increase in nonproteic solutes such as tissue lactate or products of cell cytolysis. This elevation of tissue osmotic pressure is

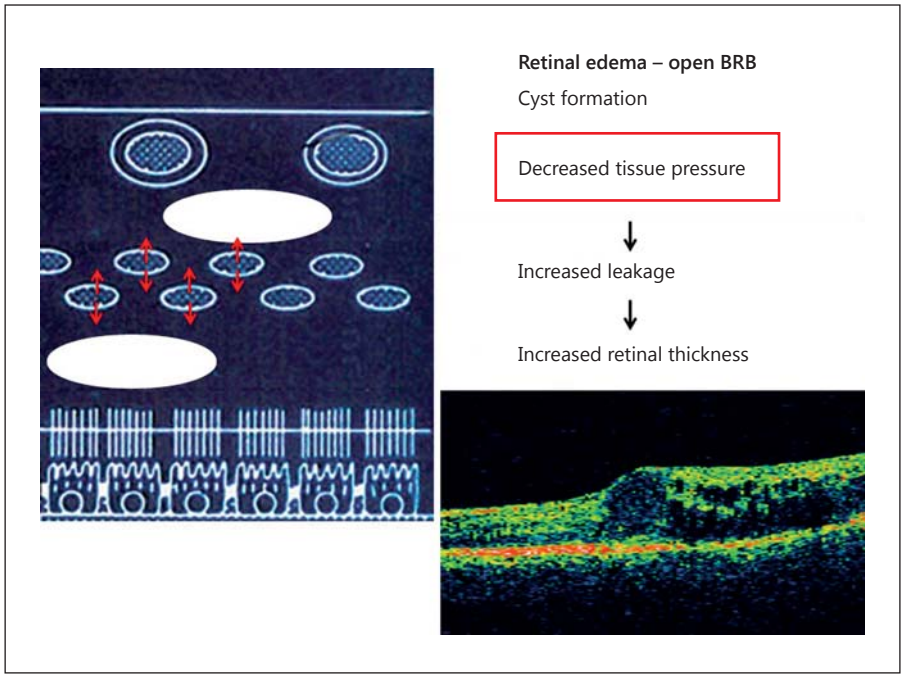

Fig. 3. Schematic drawing of the retina in a situation of retinal edema where decreased tissue pressure and increased compliance play a major role in abnormal fluid accumulation. BRB, blood-retinal barrier.

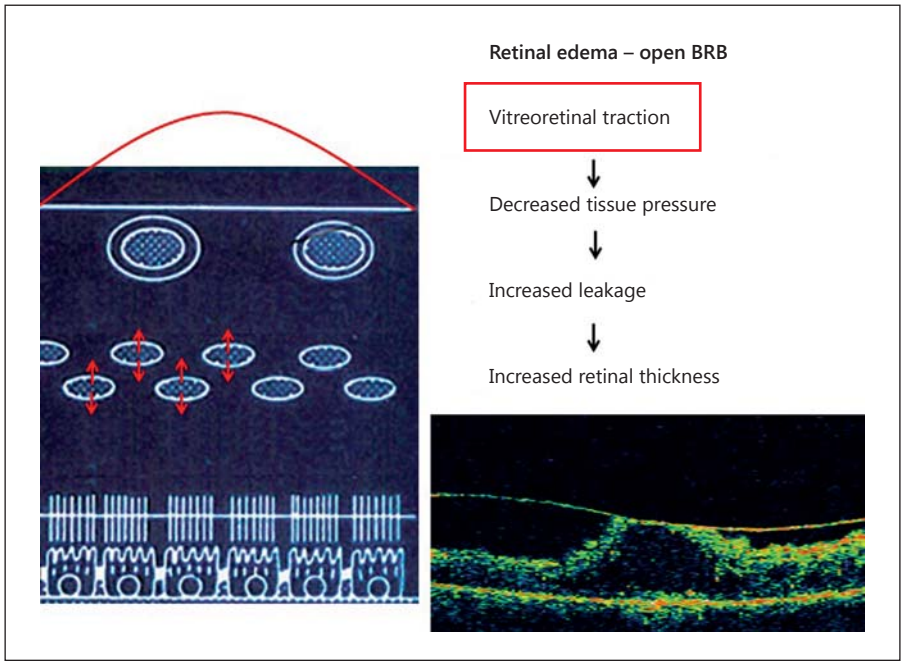

Fig. 4. Schematic drawing of the retina in a situation of retinal edema where vitreoretinal traction contributes to decreased tissue pressure and abnormal collection of fluid in the retina. BRB, blood-retinal barrier.

a major contributor to increased capillary filtration and abnormal accumulation of fluid in the retina (Fig. 5).

(5) Plasma osmotic pressure: In the brain, for edema to develop it is necessary to have a rapid reduction of 35 mOsm in plasma osmolarity. The threshold for edema formation in the retina may reflect the low compliance of retinal tissue within the closed eye.

In summary, retinal edema can develop when the rate of capillary filtration exceeds the rate of fluid transport out of the retina. Abnormal accumulation of edema fluid depends mainly on tissue compliance and changes in tissue osmotic pressure.

Although the tight junctions of the retinal endothelial cells and the RPE are the main barriers protecting and

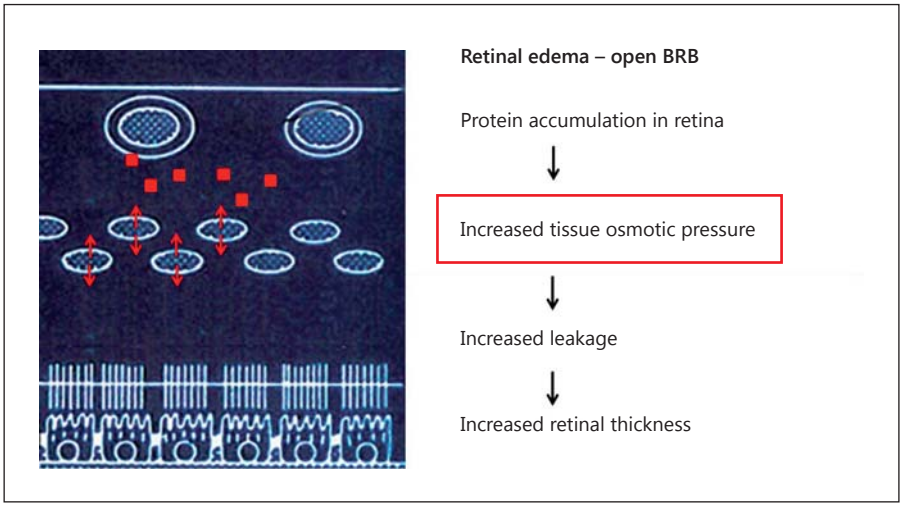

Fig. 5. Schematic drawing of the retina in a situation of retinal edema indicating how abnormal protein accumulation may contribute to increased tissue osmotic pressure and fluid accumulation. BRB, blood-retinal barrier.

modulating the microenvironment of the retinal cells, other structural features of the retina function as relative barriers to water and protein movement and are relevant to the formation of edema. The retinal extracellular space as a whole limits the free flow of water to a degree at which water leaves the eye across the RPE [18]. The normal RPE is capable of pumping a lot more water per minute than actually leaves the eye under normal conditions [19], because water cannot exit through the retina any faster. The ILM consists only of matrix material and condensed vitreous collagen, and is probably not a significant impediment to water or solute movement [20]. The intercellular pathway from the vitreous to the subretinal space is convoluted, and it ends with a band of zonulae adherentes that form the ELM. The zonulae adherentes of the ELM are less tight than the zonulae occludentes of the inner and outer BRB, but they limit the movement of large molecules larger than albumin. The rate of albumin movement across the retina is substantial, accounting for 4–5% of the difference in concentration across the retina per hour [21].

These observations have relevant implications for retinal edema. Large molecules do not diffuse freely and are blocked partially by the ELM. Thus, protein that is released within the retina will tend to remain for a period of time and will tend to back up behind the ELM. To the extent that protein is retained, water will also be retained osmotically.

In a normal eye, both passive and active forces work to move water across the retina and out of the subretinal space. First, intraocular pressure is continuously pushing water into the retina. Second, choroidal osmotic pressure draws water towards the choroid. The 2 forces are strong enough to keep the retina in place and the subretinal space dry without the intervention of active RPE transport [11].

Active transport across the RPE is needed mainly because of the requirement for a protein-free neural environment which dictates that the BRB on the RPE side is necessary for the RPE tight junctions to impede the passive absorption of fluid. Active transport is necessary to remove water that percolates through the retina from intraocular pressure and perhaps also as a safety mechanism against fluid accumulation in disease.

It is, therefore, likely that almost any retinal disorder which involves metabolic dysfunction or choroidal vascular insufficiency will alter RPE transport to some degree. A diffuse diminution of RPE transport may explain why subretinal fluid accumulates and persists in a disease such as central serous chorioretinopathy, where there is only a small focal source of fluid entry [22].

It is important to ask how extracellular fluid can accumulate in a thin tissue such as the retina with no barriers against water escaping into the vitreous. One of the answers must be the abnormal presence of proteins. In diseases with breakdown of the BRB, albumin and other proteins will be forced by blood pressure and diffusion gradients into the extracellular space of the retina. At the ILM, protein will leave the retina freely, but at the ELM, it will tend to back up, creating a localized increase in oncotic pressure, binding water and creating conditions for retinal edema. The accumulation of fluid will be greater near the sites of leakage (layers where the retinal vessels are located) and at sites of protein buildup (ELM). The active transport located at the RPE, on the other hand, is able to transport water, counteracting the protein effect.

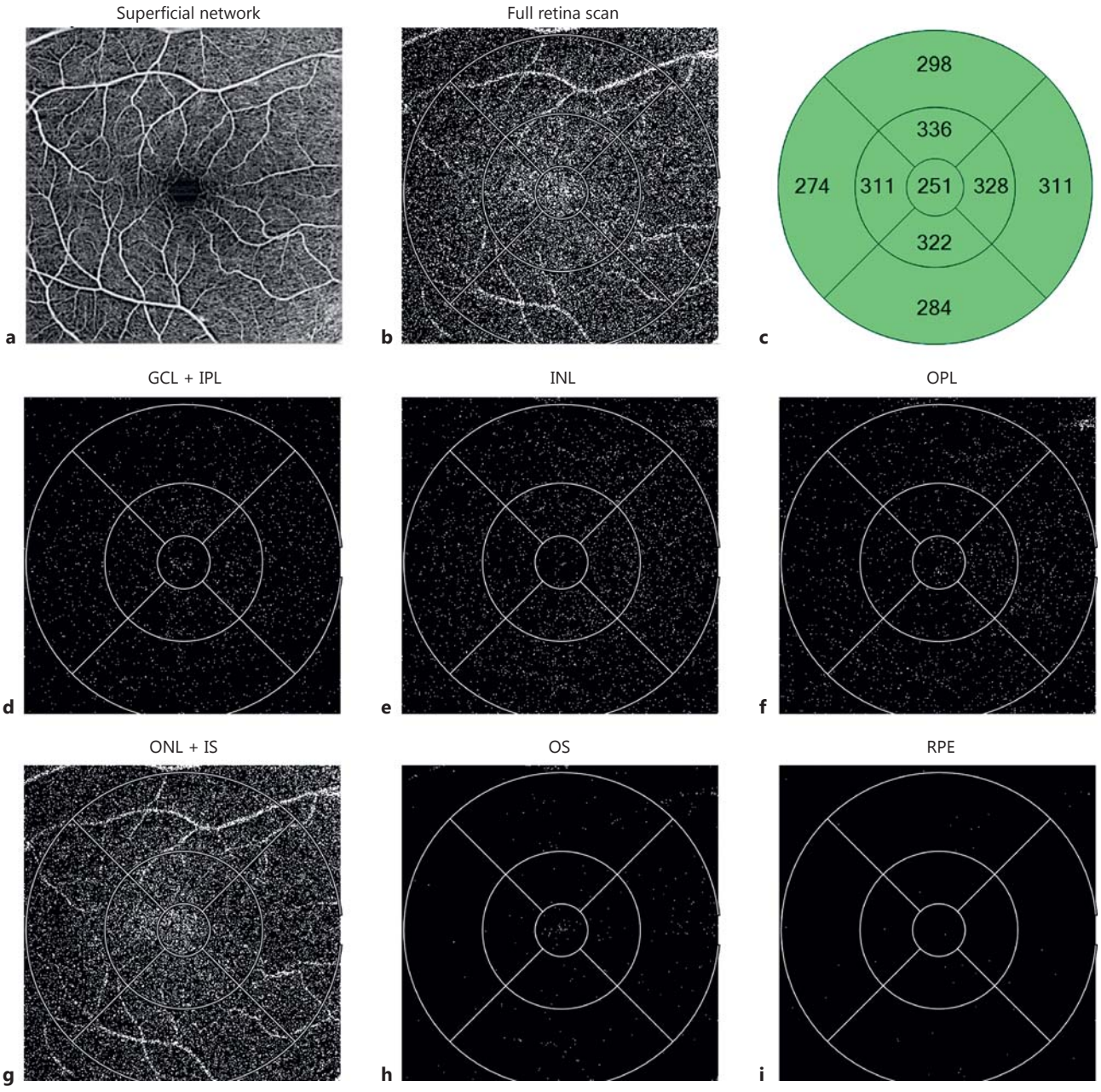

Fig. 6. OCT-Leakage maps of the right eye of a healthy subject for the full retina scan and for each of the segmented retinal layers. **a** Superficial vascular network acquired with the AngioPlex system. **b** Full retina scan. Low optical reflectivity (LOR) map. **c** ETDRS grid of retinal thickness obtained with a Cirrus 5000 device. **d–i** LOR maps for the ganglion cell layer and inner plexiform layer (GCL + IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer and photoreceptor inner segments (ONL + IS), photoreceptor outer segments (OS), and retinal pigment epithelium (RPE), respectively. Locations of LOR are identified in white. The ETDRS grid is centered at the fovea.

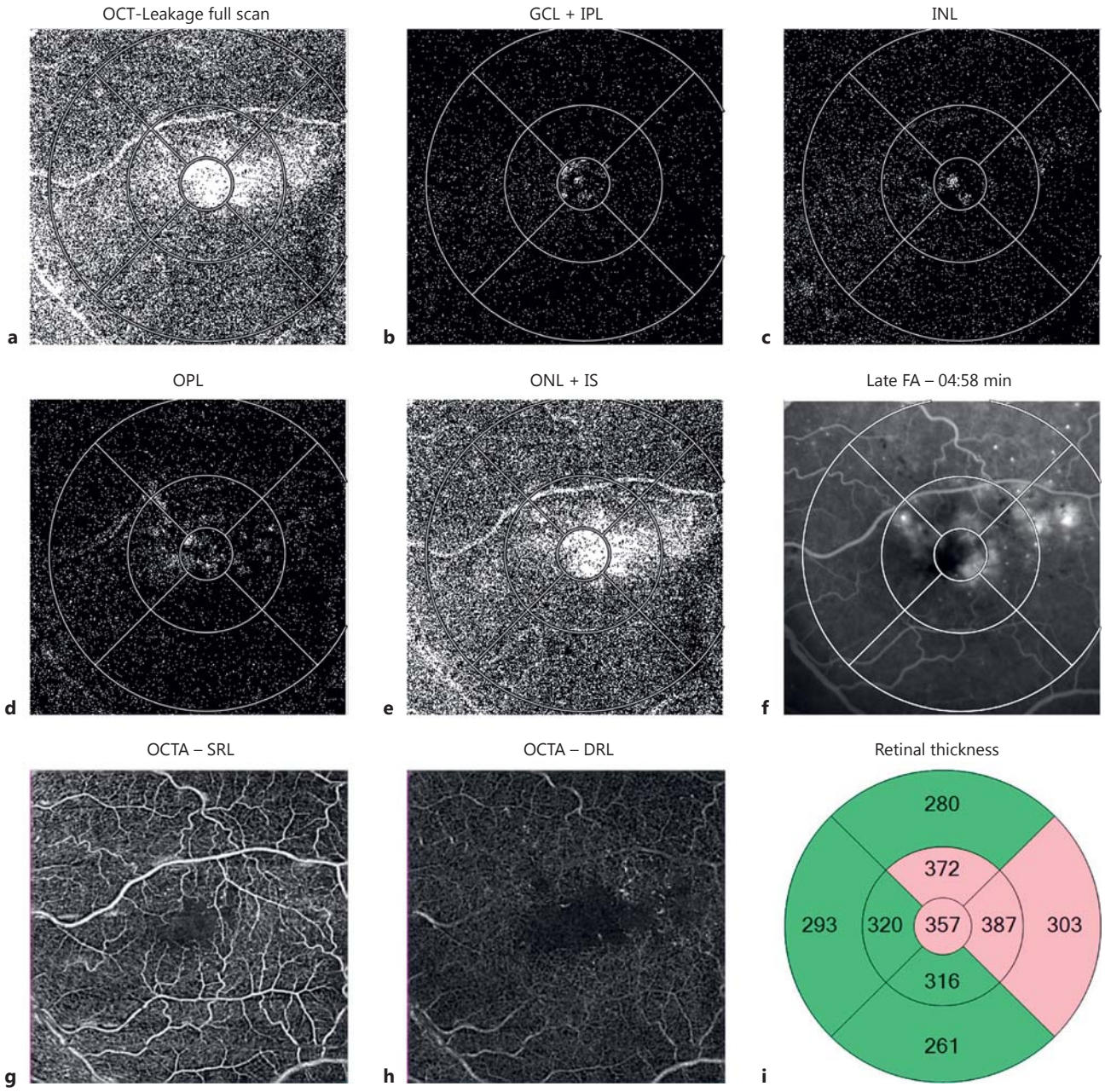

Fig. 7. Eye with diabetic macular edema and evidence of localized leakage on fluorescein angiography (FA) corresponding well with the increase in extracellular space detected in the OCT-Leakage maps of the full retina scan (**a**) and of different retinal layers (ganglion cell layer and inner plexiform layer [**b**, GCL + IPL], inner nuclear layer [**c**, INL], outer plexiform layer [**d**, OPL], and outer nuclear layer and photoreceptor inner segments [**e**, ONL + IS]) and with vascular abnormalities of the deep retinal vascular layer. **a–e** OCT-Leakage maps of the different retinal layers showing different levels of involvement but corresponding to fluorescein leakage as detected by FA. **f** FA image showing the location of fluorescein leakage. **g** OCT angiography (OCTA) image of the superficial retinal vascular layer (SRL). **h** OCTA image of the deep retinal layer (DRL) showing microaneurysms and other vascular changes in the same location of the increased extracellular space (OCT-Leakage maps) and fluorescein leakage (FA). **i** OCT thickness map showing the zone of increased retinal thickness.

Studies using methods for analyzing retinal thickness have shown relatively poor correlations between fluorescein leakage and retinal thickness in some situations. Some sites of fluorescein leakage lead to little fluid accumulation and thickness, whereas other sites of alteration of the BRB evidenced by fluorescein leakage are associated with situations of great and prolonged fluid accumulation. The degree of alteration of the BRB, allowing less or more protein leakage into the retina, may be the relevant factor.

Clinical Evaluation of the BRB

Fluorescein angiography (FA) permits a dynamic evaluation of extracellular fluid changes resulting from retinal circulatory disturbances and was the first method for demonstrating, in a clinical setting, those sites of fluorescein leakage indicating the location of an alteration of the BRB. Its reproducibility, however, depends on the variable quality of the angiographic procedure.

With the development of vitreous fluorometry, a method that was developed to quantify fluorescein leakage, a large number of clinical and experimental studies have demonstrated the major role played by alterations of the BRB as a posterior segment disease [23]. More recently, confocal retinal leakage mapping was introduced to identify sites of BRB breakdown. However, all these methods require an intravenous injection of fluorescein, which can cause nausea, vomiting, and, rarely, anaphylaxis [16].

An OCT-based method has recently been described by our group, designated OCT-Leakage, which is capable of noninvasively identifying and quantifying sites of alterations of the BRB by mapping sites of lower-than-normal optical reflectivity, thus reflecting changes in the retinal extracellular fluid. Extracellular fluid distribution in the retina is represented on OCT by the distribution of sites of lower-than-normal optical reflectivity. Increases or decreases in the extracellular fluid distribution of a given area of the retina can, therefore, be measured by the ratio of sites of low optical reflectivity (LOR) identified in the area under evaluation (Fig. 6, 7).

OCT-Leakage, using a proprietary algorithm to identify sites of lower-than-normal optical reflectivity, reliably locates and quantifies increases in extracellular space in retinal disease, showing that changes in the retinal extracellular space correlate well with the occurrence and degree of retinal edema [24].

A coregistration procedure allows mapping fluorescein leakage locations identified on FA images onto OCT data, so that locations of OCT LOR and leakage can be compared (Fig. 2, 3). There is good correspondence between locations of increased LOR area ratios and sites of fluorescein leakage on FA. The changes in extracellular space represented by the LOR area ratio corresponded well with the main sites of leakage on the FA examinations (Fig. 3). Similar correlations were found for all eyes with nonproliferative diabetic retinopathy and other retinal diseases examined by FA and the OCT-Leakage method.

It is to be noted that LOR ratios identify the main sites of leakage and also the areas of late leakage shown on FA. Furthermore, with OCT-Leakage, areas of abnormal fluid accumulation can be identified in specific retinal layers, demonstrating different involvement of different retinal layers in different eyes. Note that similar degrees of fluorescein leakage are associated with different degrees of extracellular fluid accumulation, supporting the view that the amount of extracellular fluid and its spread to the different retinal layers will be a better indicator of the severity of BRB breakdown.

Recently, OCT angiography has become available, replacing much of the information yielded by FA in a noninvasive manner [25, 26]. However, OCT angiography cannot visualize leakage, i.e., alterations of the BRB. The method here described, OCT-Leakage, is able to identify locations of increases in extracellular fluid in the different layers of the retina, and is thus capable of identifying retinal cells that are affected more than others [24]. The complementarity of the 2 methods seems therefore to potentially be of great interest for the diagnosis and management of several retinal diseases in which the presence and amount of fluid, as a marker of severity or activity, is paramount to treatment and management decisions in clinical practice.

In conclusion, retinal edema develops not only because there are protein and fluid entering the extracellular space, but also because the retinal stroma and the ELM limit clearance of large, osmotically active molecules. These molecules bind water osmotically and cause edema. Most diseases causing macular edema involve a degree of damage to both the inner and the outer BRB. OCT-Leakage is expected to help identify fluid movement in the retina and to contribute to a better understanding of retinal edema.

Finally, any therapeutic strategy for retinal edema needs to address BRB breakdown in order to impede fluid and protein leakage into the retina and take into consideration the structural changes in the retina associated with specific involvement of the different retinal layers that condition protein and water movement out of the retina and the fluid-absorptive transport of water at the RPE.

Acknowledgments

The author thanks his colleagues and collaborators in the many publications that he was privileged to share with them.

Disclosure Statement

No conflicting relationship exists.

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