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Utility of Pars Plana Vitrectomy with Internal Limiting Membrane Dissection, in the Surgical Treatment of Macular Hole and Diabetic Macular Edema. Clinic-Pathological Correlation.

TESIS DOCTORAL

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CERTIFICA: Que el trabajo titulado "**Utility of Pars Plana Vitrectomy with Internal Limiting Membrane Dissection, in the Surgical Treatment of Macular Hole and Diabetic Macular Edema. Clinic-Pathological Correlation."** ha sido realizado bajo mi dirección por el licenciado D. João Paulo Castro de Sousa y se encuentra en condiciones de ser leído ante el tribunal para optar al grado de Doctor.

Dr. Borja Corcóstegui

Barcelona, junio de 2005

El Doctor Ricardo P. Casaroli Marano, Profesor Titular de Biología Celular de la Facultad de Biología de la Universidad de Barcelona

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Aos meus Pais, a quem devo tudo.

À Beatriz e à Mafalda... pela simplicidade e pelo tempo que não lhes dediquei.

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A todos que no cité y que transporto en mi corazón...gracias.

INDICE

ABREVIATTURES

APE	Autologous plasmin enzyme
BRB	Blood-retinal barrier
BSS	Balanced salt solution
CDME	Chronic diabetic macular edema
CMT	Central macular thickness
CSME	Clinically significant macular edema
CSDME	Clinically significant diabetic macular edema
DME	Diabetic macular edema
DR	Diabetic retinopathy
ELM	External limiting membrane
ERM	Epiretinal membrane
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	Fluorescein angiography
FP	Fundus photography
HRT	Heidelberg retinal tomograph
HFF	Hole form factor
ICG	Indocyanine green
ILM	Internal limiting membrane
LM	Light microscopy
MHI	Macular hole index
OCT	Optical coherence tomography
PBS	Phosphate buffered saline
PDR	Proliferative diabetic retinopathy
PHM	Posterior hyaloid membrane
PPVP	Posterior precortical vitreous pocket
PPV	Pars plana vitrectomy
PVD	Posterior vitreous detachment
RPE	Retinal pigment epithelium
RTA	Retinal thickness analyser
TEM	Transmission electron microscopy
VA	Visual acuity
VEGF	Vascular endothelial growth factor

I. JUSTIFICATION

When Machemer introduced vitrectomy, it sounded shocking to ophthalmologists who were taught not to violate the vitreous. Similarly dramatic was Zivojnovic´s initial advocacy of retinotomy. Now surgeons intentionally remove the innermost layer of the retina. The first two of these revolutionary steps allowed treatment of conditions otherwise untreatable. Internal limiting membrane peeling is not claimed to cure pathologies for which no other treatment exists, but it has been reasonably proven to significantly increase the success rate in macular hole surgery and may be expected to do so for other forms of traction maculopathy. In this study, the intention was the evaluation of the surgical success rate of this technique in two different macular diseases, isquemic and non-isquemic macular disorders, and the evaluation of and hypothetical correlation between the clinical results and the histological analysis of this translucent retinal structure.

II. INTRODUCTION

A – VITREOUS and RETINAL STRUCTURE

A-1. Anatomy of the Retina

The innermost layer of the eye is a delicate, diaphanous tissue. It measures about 0.1 mm in thickness at the ora, 0.2 mm at the equator, and 0.56 mm adjacent to the optic nerve head. The internal aspect of the retina is in contact with the vitreous body and its external aspect is adjacent to the retinal pigment epithelium, separated by a potential space, the intraretinal space. Posteriorly, all retinal layers except for the nerve fiber layer terminate at the optic nerve head. Peripherally, the sensory retina extends to the ora serrata where it is continuous with the non-pigmented ciliary epithelium of the pars plana. Although the retina conforms to the shape of the adjacent pigment epithelium and underlying sclera, it is firmly attached to the pigment epithelium in only two areas: the optic disc and the ora serrata.

A-1.1. The Central Retina

The central retina (area centralis) or macular region is defined histologically as that area of the posterior retina having at least two layers of nuclei in the ganglion cell layer. Grossly and clinically (ophthalmoscopically), the border of this area is less clearly defined. The fovea is a 1.5 mm diameter zone centred about 4 mm temporally and 0.8 mm inferior to the centre of the optic nerve head. The inner retinal surface of the fovea is concave due to thinning of the inner retinal layers; the average retinal thickness of the fovea is about 0.25 mm, roughly half that of the adjacent posterior retina. The nerve fibber, ganglion cell, and inner plexiform layers are absent from the fovea. The inner nuclear layer is reduced to a double row of cells at the edge of the fovea and is absent within the fovea. The central 0.5 mm diameter photoreceptor layer of the fovea, the foveola, is composed entirely of cones. The blood vessels in this part of the retina are almost entirely capillaries, and the central capillary-free zone in the macula is about 0.4 mm in diameter.

The parafoveal central retina is about 0.5 mm in width and surrounds the fovea. It is characterized by the prominent cellularity of the inner retinal layers, especially the inner nuclear and ganglion cell layers. The nerve fibber layer is also relatively thick, especially in the so-called papillo-macular bundle at the nasal margin. The cone/rod ratio is 1:1.

The perifoveal retina, 1.5 mm in width, is the peripheral zone of the macular region. It extends out about 2.75 – 3 mm from the centre of the fovea where the ganglion cell layer is reduced to the single layer of nuclei seen elsewhere in the peripheral retina. The cone/rod ratio here is 1:2.

A-1.2. The Peripheral Retina

The peripheral retina gradually becomes attenuated as it approaches the ora serrata, where it terminates and becomes continuous with the non-pigmented epithelium of the pars plana. The ora serrata measures 2.1 mm wide temporally and 0.7 to 0.8 mm wide nasally. The term ora serrata refers to the serrated appearance of this zone, with so-called dentate process or teeth encroaching anteriorly on the pars plana of the ciliary body, and intervening bays that represent posterior extensions of the pars plana. The ora serrata is located more anteriorly on the nasal than the temporal side; the nasal ora, is about 6 mm posterior to the limbus and the temporal ora about 7 mm posterior to the limbus. As a rough external guide, the locations of the insertions of the rectos muscles are quite close to the ora, except for the superior rectos insertion, which varies from 7 to 7.7 mm posterior to the limbus, and therefore frequently posterior to the ora. The equator is located 6 to 8 mm posterior to the ora, and the macula is 18 to 20 mm posterior to the equator. The average distance from the ora serrata to the optic nerve is 32.5 mm temporally, 27 mm nasally, and 31 mm superiorly and inferiorly.

A-2. Histology of the Retina

The general features of retinal histology are well known from classic works by Ramón y Cajal, Polyak, and more recently, Hogan. Recent advancements in electron microscopy, immunocytochemistry, and intracellular peroxidase injections have helped determine the synaptic connections within the retina and have led to a better understanding of the functional architecture of the retina. The neurons of the retina are divided into three layers: (1) the most external (the outer, or scleral, layer) is the photoreceptor cell layer, which includes the outer and inner segments and the layer of photoreceptor cell bodies (or outer nuclear layer), (2) the layer of intermediate neurons (or inner nuclear layer), and (3) the layer of ganglion cells. The synapses are confined to the two synaptic, or plexiform, layers – the outer and inner plexiform layers.

The receptor layer of the retina is intimately apposed to the pigment epithelium; light rays pass trough the ganglion cells and inner layers to reach the photoreceptor cells, where light is transformed into an electrochemical event. The elongated axonal processes of the photoreceptor cells synapse in the outer plexiform layer with the processes of the bipolar cells and horizontal cells, whose cell bodies are located in the inner nuclear layer. The horizontal cells form a means of lateral communication between photoreceptor cells, among which they send processes. The bipolar cells synapse with amacrine and/or ganglion cells. Amacrine cells are located primarily in the vitreal portion of the inner nuclear layer. They extend processes to adjacent amacrine or bipolar cells, and their axons synapse with ganglion with ganglion cells. The axons of ganglion cells converge to form the nerve fibber layer and exit the eye as the optic nerve. Another cell type, whose perikarya, is the inner plexiform cell. Unlike amacrine cells, however, the inner plexiform cells extend processes into both synaptic layers.

The external (outer) limiting membrane is formed by junctional complexes between cell membranes of the major glial cells, the Müller cells, and photoreceptor inner segments. The internal (inner) limiting membrane consists of a basement membrane, which is actually a surface modification of the vitreous body, and the expanded vitreal processes of Müller cells. Within the retina, fine processes of Müller cells envelop, at least partially, all the neurons. These processes extend laterally from the main portion of the Müller cells, which extend from the external to the internal limiting membrane as radial fibbers.
In conclusion, the histological layers in the human retina, from vitreous (inside) to choroid (outside), are (Figure 1): a) internal or inner limiting membrane (ILM), b) nerve fiber layer (NFL), c) ganglion cell layer (GCL), d) inner plexiform layer (IPL), e) inner nuclear layer (INL), f) outer plexiform layer (OPL), g) outer nuclear layer (ONL), h) external or outer limiting membrane (OLM), i) photoreceptors layer (inner and outer segments) (RCL), j) retinal pigment epithelium (RPE).

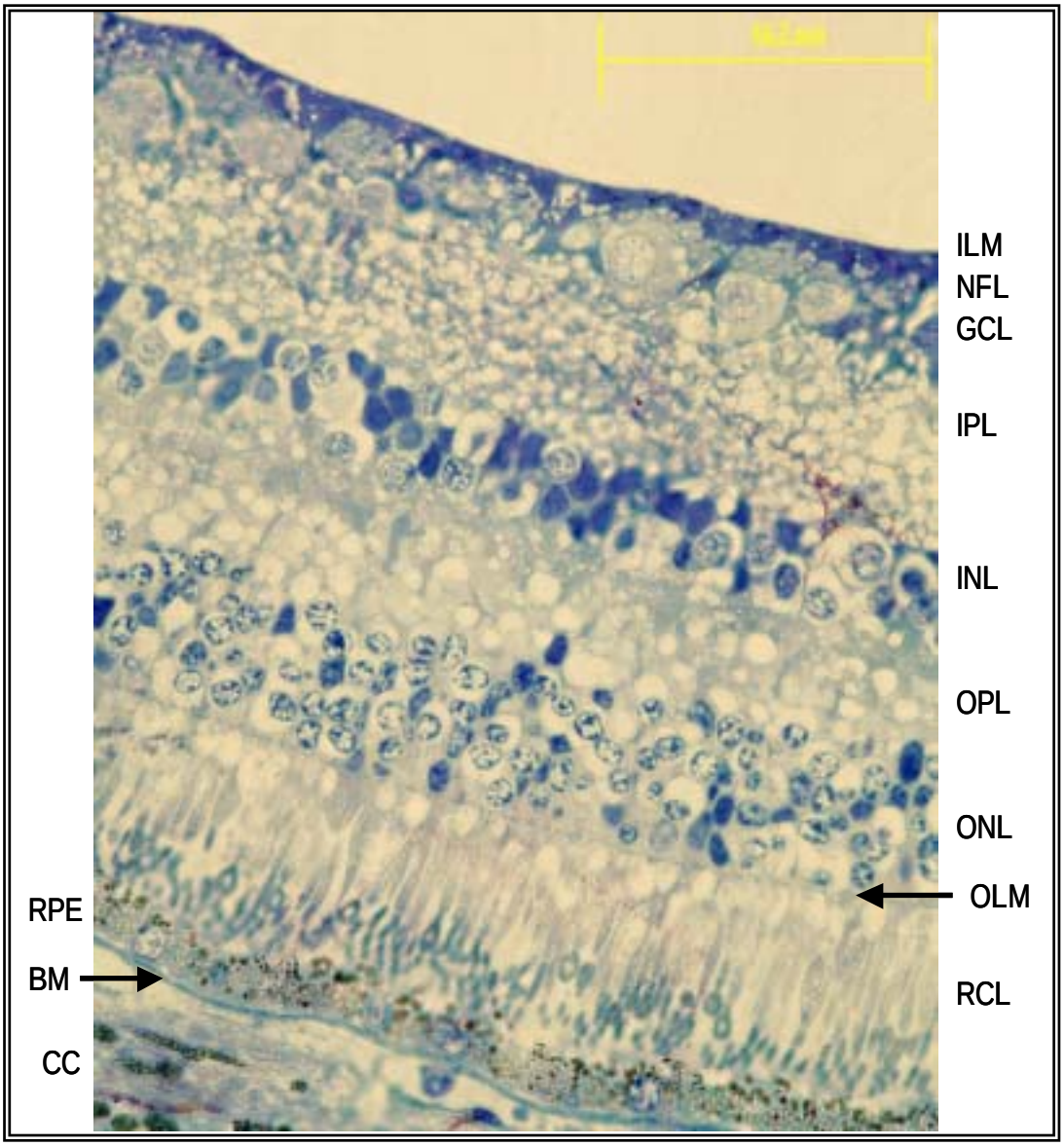

Figure 1 - Histological aspect observed in one normal human retina of the study, with the vitreous face (up) and the choroidal face (down, left side). ILM: inner limiting membrane; NFL: nerve fiber layer; GCL: ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer ONL: outer nuclear layer; OLM; outer limiting membrane; RCL: photoreceptors layer; RPE: retinal pigment epithelium; BM: basal membrane; CC: chorio-capillar.

A-3. The Vitreous

The vitreous body occupies four-fifths of the globe. It has an average volume in the adult eye of 4 cc and an average weight of 4 grams. The anterior vitreous border is a concavity centrally. The concavity is occupied by the lens and is called the patellar fossa. At the periphery of the concavity, the vitreous surface is attached to the posterior capsule of the crystalline lens along a roughly circular zone approximately 1 mm in width and 8 to 9 mm in diameter. This zone of attachment is known as the hyaloideo-capsular ligament. The attachment is strong in children and young adults but weakens with advancing age. It encloses a potential space centrally between the lens and vitreous, the retrolental space of Berger. Posterior to the lens-zonular diaphragm, the peripheral vitreous at birth is in contact with the pars plana, retina, and optic nerve. It shape is roughly spherical, conforming to the shape of the ocular structures with which it is in contact. The peripheral 100 microns or so of the vitreous consists of a relatively dense array of collagen fibrils in comparison with the central vitreous, and the peripheral portion is known as the vitreous cortex.

There are several zones of firm attachment between the vitreous cortex and the inner retinal surface in the young eye. The strongest of these is along the vitreous base, a circular band straddling the ora serrata and varying in width from 2 to 6 mm. The attachment of vitreous cortex at the vitreous base remains strong throughout life, and traction on this area of the vitreous will be transmitted to the peripheral retina and pars plana. Another attachment between vitreous and adjacent structures occurs in a circular zone at the margin of the optic nerve head. With age, this attachment weakens relative to that at the vitreous base, but it may be the last area to separate during creation of a posterior vitreous detachment. In an eye with a total posterior vitreous detachment, the circular zone of vitreous that was adherent at the optic nerve head may be visible within the vitreous cavity as an annular opacity known as Weiss ring. The vitreous is relatively firmly adherent to the retina along the course of the major retinal vessels. Vitreous traction along the vessels may give rise to partial thickness retinal breaks or full-thickness breaks with a bridging to retinal vessels. Finally, a loose vitreomacular attachment is present.

A-3.1. The Vitreoretinal Juncture

The vitreous cortex is a gel-like structure composed of collagen fibrils embedded in a watery milieu rich in hyaluronan and other glycosaminoglycans. The collagen fibrils in the vitreous are heterotype: 80% are formed from type II collagen, but types V, XI, VI and IX are also present (Ferris, 1997). The posterior vitreous cortex is formed by a condensation of these collagen fibrils. The biochemical structure of mammalian vitreous was recently reviewed by Bishop (Bishop, 1996): the predominant cell type in the vitreous is the hyalocyte; fibroblasts account for approximately 10% of the cellular population.

The exact nature of the vitreoretinal juncture is a subject of ongoing debate (Ferris, 1997). Zimmerman and Straatsma (Zimmerman et al, 1960) have described the existence of fine, fibrillar attachment

between the posterior vitreous cortex and the ILM, and they claimed that these were responsible for the extremely intimate union between normal retina and vitreous. Attachment plaques between the Müller cells and the ILM have been described in the basal and equatorial zones of the fundus but not at the posterior pole, except at the fovea. It is generally accepted that this adhesion between the vitreous and the retina, whatever its nature, weakens progressively with age. These age-related changes in the vitreoretinal interface, were investigated by Sebag and associates (Sebag et al, 1991) using dark-field slit microscopy, scanning, and transmission electron microscopy. In 40% of the specimens examined from persons younger than 20 years of age, the ILM was shown to adhere to the posterior vitreous cortex by diffuse sheet-like adhesions that encompassed the macula and peri-papillary region. Sebag suggests that these adhesions result from the extra-cellular matrix components between the type II collagen of the vitreous cortex and the type IV collagen of the ILM.

A-3.1.1. Posterior Hyaloid Membrane

Conventional teaching states that during posterior vitreous detachment (PVD), the posterior vitreous cortex separates from the ILM to form the posterior hyaloid membrane (PHM) (Duke-Elder, 1969; Balazs et al, 1961). There may also be an accompanying Weiss ring, signifying detachment of the vitreous from the peri-papillary region. The extent of the PVD may be difficult to ascertain by slit-lamp biomicroscopy alone; kinetic B-scan ultrassonography and, more recently, laser biomicroscopy and the OCT have also been used to assess the focal areas of PVD and vitreoretinal traction (Ferris, 1997). Scanning electron microscopy, confirms that the PHM is a definitive structure with an interwoven fibrillar architecture that distinguish it from posterior vitreous cortex.

*A-3.1.2. The Internal Limiting Membrane of the Retin*a

The internal limiting membrane (ILM) of the retina traditionally represents the basement membrane of the Müller cells composed of type IV collagen, laminin, and fibronectin, and the remaining inner portion is formed by condensed vitreous fibrils and mucopolysaccharides (Balazs et al, 1964). It is a multilaminar structure with a lamina rara interna, which is adjacent to the Müller cells, a lamina densa, and a lamina rara externa (Figure 2). The ILM is of variable thickness (Foos, 1972), thicker posteriorly (0.5 – 3.2 µm) and becoming markedly thinner over the fovea (0.01 – 0.10 µm), at the disc and over the major retinal vessels. It also thickens with age (Duke-Elder, 1969), which suggests that it may be continuously laid down throughout life. Fibronectin and laminin have been found in human ILM (Jerdan et al, 1989), although the significance of these findings in the pathophysiology of vitreoretinal disorders remains unclear.

Unlike the posterior vitreous cortex, the ILM has type IV as the predominant type of collagen, not type II (Jerdan et al, 1989). Type IV collagen, along with laminin, acts as the scaffold for basement membrane formation and is a unique intrinsic structural component of basement membranes

(Yurchenco, 1990; Kuhn et al, 1985). The work of Constable and colleagues (Constable et al, 1981) suggests that the ILM in dogs is capable of regeneration. However, the only evidence of ILM regeneration in the human eye was seen at autopsy in an eye that had previously undergone removal of an epiretinal membrane with ILM.

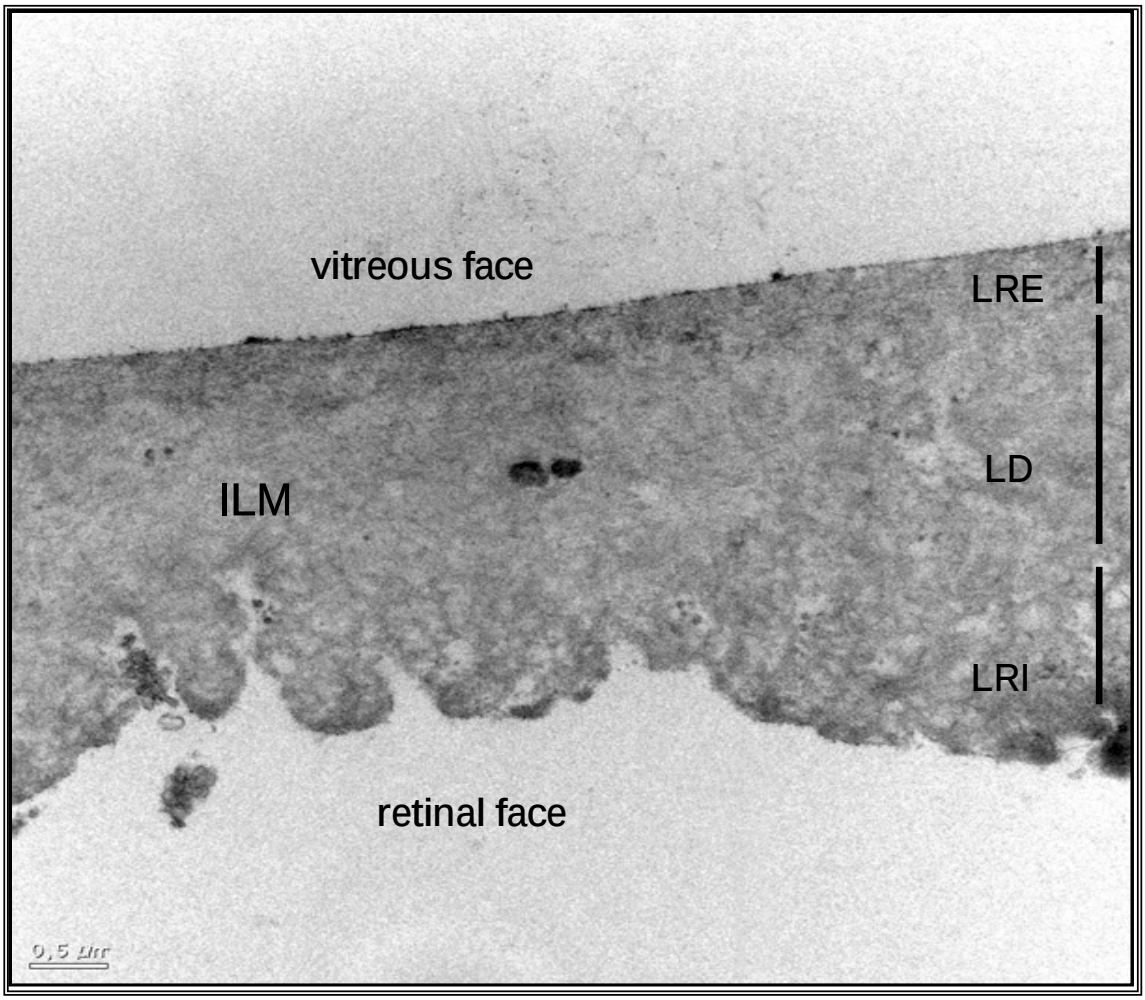

Figure 2 – ILM of a normal human retina, with it undulated face (retinal) and it smooth face (vitreous). Laminar structure of the ILM: the lamina rara interna (LRI), the lamina densa (LD) and the lamina rara externa (LRE).

The outer (vitreal) surface of the ILM appears smooth, but the ILM has a rough, undulated inner (retinal) surface (Figure 2). This morphologic difference is most pronounced at the posterior pole and becomes less apparent the further one goes anteriorly. The ILM is adherent to the type II collagen fibers of the vitreal cortex. This adhesion is marked at the posterior pole and is induced mainly by several extra-cellular matrix molecules. As a result, vitreal tractional forces can be transmitted to the retinal surface, contributing to the pathogenesis of several “traction maculopathies” such as macular pucker, macular hole, or vitreoretinal traction syndrome. This traction is caused by remnants of collagen fibers of the vitreous cortex after posterior vitreous detachment (PVD), or vitreoschisis, which

is a split of the vitreous cortex during PVD due to extremely strong adhesions of the ILM and the vitreal collagen fibers.

The structural boundary between the retina and the vitreous consists of the ILM, which is adherent to the collagenous cortex of the vitreous on its one side, and to the Müller (glial) cell end-feet on the other (Fine, 1961). This latter adhesion is strong enough to cause tearing between the end-feet and the rest of the Müller cells if the ILM is peeled from the isolated chicken (Halfter et al, 1987) and mouse retina (Kroger S, personal communication, 2002; Wolf et al, 2004). Removal of the end-feet must cause severe changes in the physiologic features of Müller cells, even if the membranes eventually seal and the cells survive (Newman, 1984). Because the Müller cells (and, in particular, their end-foot membranes) are known to be sites of an intense exchange of waste products of active retinal neurons (e.g. potassium ions and protons) with the vitreous body acting as a buffering sink (Newman, 1996), any loss of Müller cell end-feet, or even of their normal interaction with the vitreous, should interfere with retinal function (Winter et al, 2000). Unfortunately, it is difficult to assess whether ILM peeling causes impairment of the human retina (Wolf et al, 2004). On the functional point of view, any measured parameters are compared with the situation before peeling, that is, when the retinal functions were already depressed. On the (ultra) structural side, the retinal tissue of a patient cannot be studied after peeling, for obvious reasons.

B – PRINCIPLES OF OPTICAL COHERENCE TOMOGRAPHY

Standard ultrasound depends on reflection of high-frequency sound waves (in the order of 10 MHz travelling at 1500 m/s in water) and is limited to a resolution of about 150 µm. OCT uses reflection of short coherence length light (usually 830 nm wavelength at a speed of 3×10^8 m/s) and results in resolution in the order of 10 µm. Although high-resolution ultrasound systems are available allowing up to 20 µm resolution, their penetration in tissue is limited to only 4-5 mm. The commercially available OCT machine from Humphrey Instruments Ltd (San Leandro, CA, USA), was developed by a team of bioengineers and ophthalmologists at the Massachusetts Institute of Technology, and is currently the only machine in clinical use. The OCT machine consists of a scanner attached to a slit-lamp, a video monitor, a computer, and a printer. Following its release in 1995, the product was rearranged as OCT 2 in 2001 when the hardware was ergonomically improved resulting in a smaller equipment footprint and the patient sitting opposite rather than perpendicular to the examiner. However, the images from OCT 1 and OCT 2 are similar. OCT 3 released in 2002, produces much higher resolution images (8 µm), and offers other features such as improved ease of operation and faster image capture and processing, but the principle by which the machine functions is unchanged (Thomas et al, 2004).

Posterior segment imaging is achieved by projecting light from a super luminescent diode via a slit-lamp and integrated + 78 D biomicroscopy lens onto the retina, and acquiring an image via a computer. The incident light beam of 200 microwatts is reflected at the interface between each ocular

structure, and the time taken for the reflection enables the distance and the relative position of the structures to be calculated by comparison to a reference beam using interferometer. The reflected signal is thus an A-scan of a small cross-section of the eye, and the intensities of the various signal returns are converted into a false-colour rainbow scale starting with black and the blue end of the spectrum, progressing through green, yellow, red to white in increasing order of reflectivity. If the axis of the scan is moved horizontally, sequential sections can be aligned to create a composite cross-sectional colour image. Typically, one posterior segment OCT 1 or OCT 2 image is composed of 100 individual colour-coded A-scans aligned with respect to the bright reflection from the retinal pigment epithelium (RPE). OCT 3 images are composed of 512 A-scans, hence yielding higher resolution. It is important to realise that each interface in the OCT image represents a difference in optical properties and not necessarily a different anatomical structure, although early reports suggest that OCT 3 may be capable of demonstrating greater anatomical detail than OCT 1 and OCT 2 (Thomas et al, 2004).

Various scan patterns can be used, including single lines of differing length and orientation (with shorter scans having a higher resolution), lines arranged radially centred on the same point, annular scans of varying diameters and sequential parallel lines. Each OCT scan is captured individually and takes about 1 second to acquire. OCT 3 can acquire up to six scans simultaneously in 1 second. The scan settings can be memorised allowing exactly the same scan position to be used on a fellow-up examination. The field of view is limited to about 30º, and although scan resolution may be limited by media opacities, it is still possible to capture an image through moderate vitreous haemorrhage or cataract.

The normal foveal thickness measured by OCT is approximately 160 µm (Hee et al, 1998; Massin et al, 2002). Early images presented from ultra-high resolution OCT technology, which is in development, appear to resolve the retina into its individual layers, including the external limiting membrane. OCT 3 has an intermediate resolution but is a significant step towards achieving a “non-invasive optical biopsy”.

With the normally clear optical media, OCT is readily applicable to ophthalmic imaging. The cross-sectional image obtained not only allows a diagnosis to be made, for example, in macular holes, pseudohole, lamellar holes (Puliafito et al, 1995; Hee et al, 1995), but since the coordinates and settings of each scan can be memorised, the same area of retina can be scanned on different occasions allowing monitoring of the condition. In addition, measurement of retinal thickness is possible allowing quantitative follow-up, for example, in diabetic macular edema. OCT measurements of retinal thickness have been found to be highly reproducible both between observers and between visits (Hee et al, 1995; Massin et al, 2002). The OCT image shows abnormalities of the vitreomacular interface and the profile of the inner retinal boundary. Intraretinal abnormalities may result from abnormal reflectivity due to changes in the optical properties of retinal tissue morphology. Hyper reflectivity in the retina or choroids may result from scar tissue, inflammatory infiltrates, hard exudates, or haemorrhages. Hypo reflectivity is usually due to fluid accumulation such as macular edema or subretinal fluid, the location of which is usually readily distinguishable and useful in the differential diagnosis of central serous retinopathy, pigment epithelial detachment, and retinoschisis (Thomas, 2004).

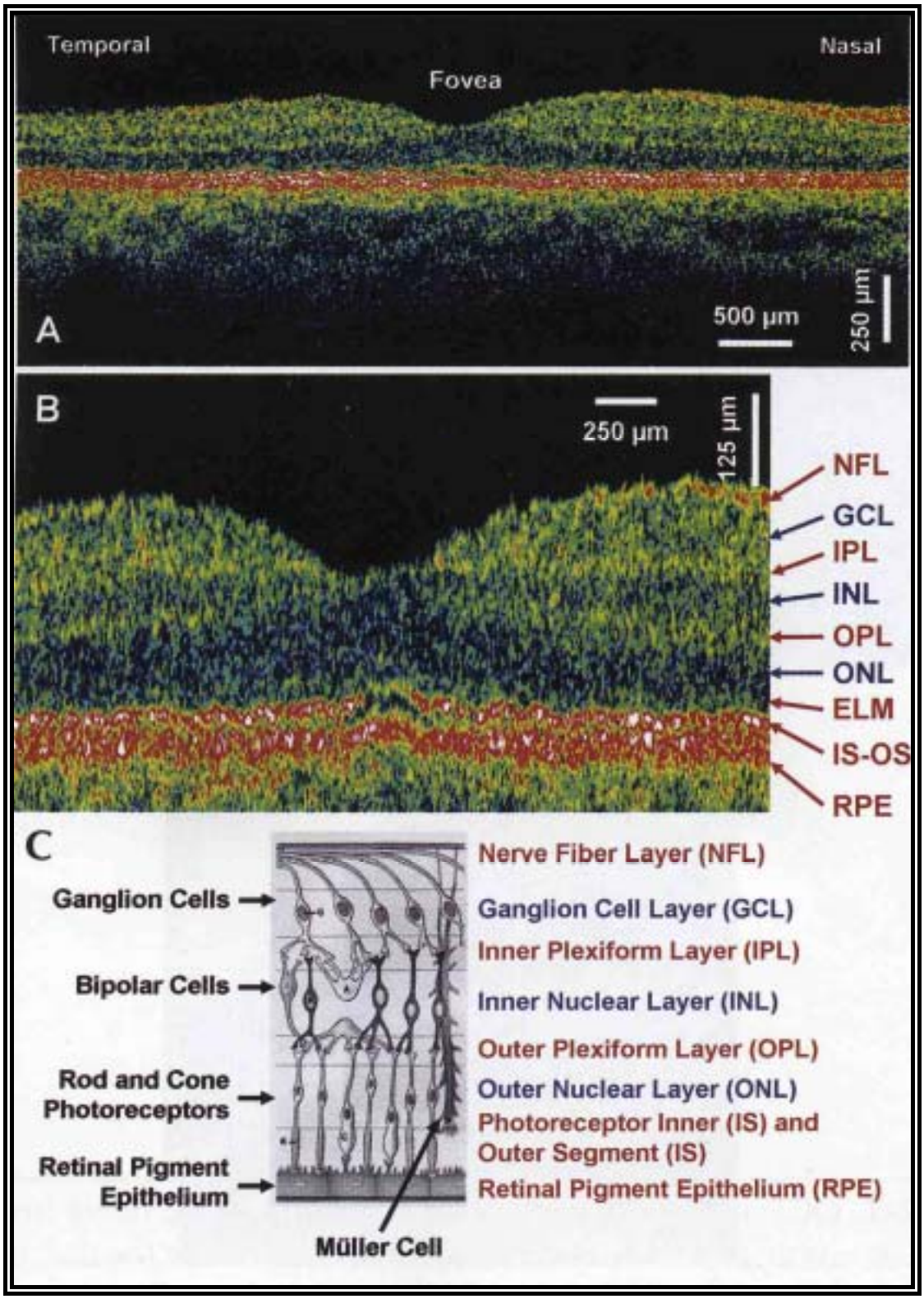

Figure 3 – OCT images (A and B) of the macular/foveal region. Morphology of the retina (C), including four cell layers and two layers of neuronal interconnections.

C - MACULAR HOLES

C-1. Definition

Macular hole is a condition in which an anatomic opening or dehiscence develops in the fovea centralis. Henry Noyes published the first clinical description of a macular hole in 1871 (Noyes, 1871). Since then our understanding of development and pathogenesis of macular holes has much improved. However, it took more than 100 years, until Kelly and Wendell reported the first successful closure of a series of macular holes by pars plana vitrectomy and membrane peeling in 1991 (Kelly et al, 1991).

C-2. Pathogenesis of Idiopathic Macular Holes

The precise pathogenesis of macular hole formation remains controversial, but it probably involves tangential and/or anteroposterior vitreofoveal traction. Macular hole formation typically evolves over a period of weeks to months through a series of stages that were first described by Gass (Gass et al, 1988; Gass, 1995). Studies investigating the nature of the "prehole" lesion proved controversial (Ezra, 2001). Morgan and Schatz (Morgan et al, 1986) suggested that involutional thinning resulted in a depressed central area at the fovea, which represented the prehole lesion, while others proposed that a foveal cyst (McDonnell et al, 1982; Morgan et al, 1986; Trempe et al, 1986) occurred before hole formation. Several underlying mechanisms were implicated by these investigators, including degenerative macular thinning, degeneration of a macular cyst, intrinsic retinal pigment epithelium disease, systemic vascular disorders, and hormonal influences (Margherio et al, 1972; Trempe et al, 1986). These early studies were retrospective in nature and consisted of lesions examined and defined by different clinicians. It was Gass and Johnson (Gass et al, 1988; Johnson et al, 1988), who described the yellow spot and ring as the prehole lesion, and the systematic clinical staging and evolution of idiopathic full thickness macular hole, based on detailed observation and follow-up. They proposed that focal shrinkage of the cortical vitreous in the area of the fovea resulted in tangential tractional forces acting on the fovea.

Full thickness macular holes (FTMH) were described in the last century, in relation to trauma (Knapp et al, 1869; Collins, 1900; Noyes, 1871), inflammation, myopia (Collins, 1900; Noyes, 1871), cystic foveal degeneration, systemic vascular disease, and mechanical forces due to fluid motion and counter currents in the premacular bursa during ocular movements (Yaoeda, 1967; Aaberg, 1970; Gass et al, 1988; Gass, 1995; Trempe et al, 1986; Morgan et al, 1986; Johnson et al, 1988) but more recent clinical studies have shown that the vast majority are idiopathic (Yaoeda, 1967; Aaberg, 1970; Gass et al, 1988; Gass, 1995; Trempe et al, 1986; Morgan et al, 1986; Johnson et al, 1988) and occur with a prevalence of 1/3300 usually in the 6^{th} and 7^{th} decades of life (McDonnell et al, 1982). Many clinical studies have implicated vitreous traction as the cause of idiopathic FTMH formation and it is

now widely accepted that traction at the level of the vitreofoveal interface is the underlying mechanism (Ezra, 2001).

Gass (1988), Johnson (1988) and Guyer and colleagues (1990; 1993) have suggested that FTMH arise from local tangential vitreofoveal traction above the fovea, due to three mechanisms: shrinkage of the prefoveal vitreous cortex caused by cellular remodelling; fluid movements and counter currents in the vitreous and by contraction of a thin glial membrane on the retinal surface (Ferris, 1997). The presence of a glial membrane has been emphasised by Gass (1995) who has proposed that in the "can-opener" stage 2 lesion, a glial membrane covers the surface of an "occult" hole and progression of the can opener represents an extending dehiscence in the membrane itself, finally resulting in a free "pseudo-operculum", comprising exclusively glial tissue, suspended on the vitreous cortex after vitreofoveal separation has occurred. The position of a "pseudo-operculum" suspended on the vitreous cortex anterior to the retinal plane is a consequence of the anterior vectorial component of a tangential force at the surface of the fovea.

More recently, Gass has emphasised the importance of the foveal Müller cell "cone" (Gass, 1999), originally described by Yamada (1969) and Hogan and associates (1971) in histological studies of the normal human foveola. These studies showed the foveola to be composed of an inverted cone of Müller glia with a truncated apex up to the external limiting membrane (ELM). Between the apex and the ELM were radially oriented inner cone segments radiating towards the beginning of the outer nuclear layer of cone nuclei. The base of the cone formed the umbo and extended into the clivus in the perifoveolar region. The internal limiting membrane (ILM) lining the base of the cone was extremely thin (10-20 nm) compared with the peripheral fovea. The sides of the cone were apposed to radiating inner segments centrally and cone nuclei in the outer nuclear layer more peripherally towards the perifoveolar region. Gass has suggested that the Müller cell cone has three important roles in idiopathic FTMH formation (Gass, 1999): (1) the glia contain concentrated superficial xanthophylls, which migrates centrifugally during the formation of a FTMH and may be seen biomicroscopically as a yellow spot or ring. The presence of xanthophylls with opercula supports this hypothesis. (2) The Müller cell cone provides structural support for the radiating inner cone segments at the foveola and its disruption may lead to damage and atrophy of the cone cells in this area. (3) The Müller cells within the cone invade the pré foveolar vitreous cortex and initiate cellular remodelling and contraction, resulting in tangential forces on the foveola, centrifugal migration of photoreceptors and xanthophylls, further disruption of the Müller cell cone, and eventually an umbo dehiscence. Furthermore, a potential cleavage plane may exist between the truncated apex of the cone and the cone elements at the umbo, which may result in schitic changes at the foveola during hole formation.

Although previous histological data from light and electron microscopic analysis of prefoveal vitreous cortex, removed at the time of surgery for impending holes, have confirmed the presence of glial cells at this early stage of hole development (Campochiaro et al, 1992; Smiddy et al, 1989), it remains unclear whether these glia initiate foveolar traction by cortical vitreous remodelling or whether they represent an attempted healing response to mechanically induced damage at the fovea due to vitreous traction (Gass, 1995; Madreperla et al, 1995; Ezra et al, 1997). A number of observations indicates that epiretinal glial proliferation at the fovea occurs commonly even in apparently normal

asymptomatic eyes: (1) Foos (1977) has demonstrated at post mortem eyes, the presence of a preretinal glial membrane in up to 30% of apparently normal eyes without PVD where no retinal distortion is present; (2) the majority of eyes (70%) with holes do not appear to have significant visible membrane around the edge of the hole; (3) only about 50% of holes are found to have glial proliferation at surgery, and in the vast majority this is of a very friable and nebulous nature rather than the typical confluent ERM found in macular pucker (Wendel et al, 1994).

Histopathological studies on the ultrastructure of stage 3 macular hole opercula have provided further clues to the patho-physiological mechanisms in FTMH formation. Overall, all opercula (100%) studied so far have been found to contain Müller cells and/or fibrous astrocytes, 61-100% have had identifiable ILM fragments and about 40-50% have contained cone photoreceptors ranging from a few scattered cones to those with densely packed cone photoreceptors (Gass, 1999; Madreperla et al, 1995; Ezra et al, 1997).

It is difficult on histological grounds to determine whether glial cells within an operculum are avulsed epiretinal glia within a membrane (that is, a pseudo-operculum), avulsed inner retinal glia (that is, a true operculum), or both. However, the presence of fragments of internal limiting membrane in some opercula (61-100%) (Madreperla et al, 1995; Ezra et al, 1997), indicates that a significant number of opercula containing only glia avulsed from the inner fovea and are also “true opercula”. The lack of identifiable ILM in some opercula suggests that inner retinal glia and that in these cases “in situ” disruption of the Müller cell cone may indeed be sufficient to cause an umbo dehiscence (Gass, 1999). The variation in cone photoreceptor density in opercula (40-50% contain photoreceptors) probably reflects the amount of foveal tissue avulsed during hole formation. In fact, clinicopathological correlation on a small number of opercula from stage 3 holes, excised during vitrectomy, has shown that the anatomical success rate following surgery is lower in cases with opercula containing a high cone density (Ezra et al, 1997). This may reflect the larger tissue defect in these cases, where effective postoperative glial repair is less likely to occur. However, it is difficult to draw firm conclusions from these data because of the small numbers of cases involved and clearly further clinicopathological correlation of macular hole opercula would be valuable but difficult owing to the small size of the opercula.

Recent studies of the early stages of FTMH using optical coherence tomography (OCT) (Hee et al, 1995; Puliafito et al, 1996; Gaudric et al, 1999) have shown recent hypotheses of the pathogenesis of FTMH formation, because vitreomacular separation may actually occur around the posterior pole before hole formation rather than after formation as suggested by Gass. These observations have suggested that an incomplete posterior pole vitreous cortical separation occurs with residual tethering at the fovea and optic disc, and assumes a “trampoline” configuration (Gaudric et al, 1999; Mori et al, 2000; Gallemore et al, 2000). This anatomical configuration may be seen in stage 1 lesions where no vitreous separation can be detected clinically by biomicroscopy and would be consistent with the observation on post-mortem eyes, using electron microscopy, of firm vitreoretinal adhesions at the fovea, which are not present elsewhere (Foos, 1972; Kishi et al, 1986) (Figure 4 a). Mechanical forces transmitted through the vitreous with eye movements and possibly contraction of the vitreous face between the fovea and the disc and between the fovea and the residual peripheral extra-macular

temporal attachment, would result in an oblique vector force with both anterior and tangential vectorial components (Chauhan et al, 2000), leading to avulsion of foveal tissue and complete posterior pole vitreous separation. As further traction occurs, a full thickness tear is seen trough the fovea resulting in the formation of a stage 2 FTMH with focal tethering of the posterior vitreous cortex to the operculum (Figure 4, b and c). Eventually, the operculum is completely avulsed from the fovea resulting in a stage 3 FTMH characterised by complete vitreomacular separation and release of the tractional forces on the fovea. Thus the operculum would come to lie anterior to the preretinal plane and this is the earliest time at which vitreous separation over the macula can be detected with biomicroscopy (Figure 4 d).

In a recent OCT study, Gaudric and colleagues (1999) confirmed this “trampoline” configuration of the vitreous cortex in the majority of eyes with FTMH. They also demonstrated that some impending holes are characterised by foveal cyst formation, with the presence of a cystic cavity beneath the elevated retina tissue beneath the cystic space. The potential cleavage plane in the foveola between the inner retinal Müller glia and the laterally displaced cone inner segments was confirmed. They concluded that in a significant number of holes, localised vitreofoveal traction results in the formation of a foveal cyst, through the cleavage plane, which later becomes deroofed. The avulsed roof, comprising glial cells only, would form a glial operculum, while the uncovered floor of the cyst, comprising cone nuclei, would degenerate resulting in a FTMH. These findings indicate that FTMH may arise from different precursor lesions, as a full thickness foveal detachment, as described by Gass, or as a foveal cyst. It is possible to speculate that in FTMH arising from cysts, the operculum would comprise glia only, while in those proceeded by a cyst, the operculum would contain some photoreceptor elements (Ezra, 2001).

Although the primum movens of macular hole formation remains unexplained, the sequence of events leading to the initial stages of this formation is now well documented. Several authors already postulated many years ago that the initial stages of macular hole formation were the result of the process of posterior vitreous detachment (McDonnell et al, 1982; Avila et al, 1983; Kakehashi et al, 1996; Trempe et al, 1986; Reese et al, 1967). More recently, Hee and colleagues (1995) showed, with OCT, that when a foveal cyst developed in the fellow eye of an eye with a macular hole, the partially detached posterior hyaloid adhered to the foveal centre. Gaudric and associates (1997) also showed that in fellow eyes of macular holes, posterior hyaloid detachment began at the posterior pole, around the macula. Haouchine and colleagues (2001) showed that in every cases of cystoid changes, regardless of whether a macular hole was present in the fellow eye, the posterior hyaloid was partially detached over the posterior pole and remained adherent only to the foveal centre, exhibiting a biconvex shape on a linear scan. In all cases of Haouchine study (Haouchine et al, 2001), whether the cystoid changes eventually resolved or evolved into a lamellar or full-thickness hole, the profile of the posterior hyaloid changed. It became either flat or convex over the posterior pole and was at impending hole stage.

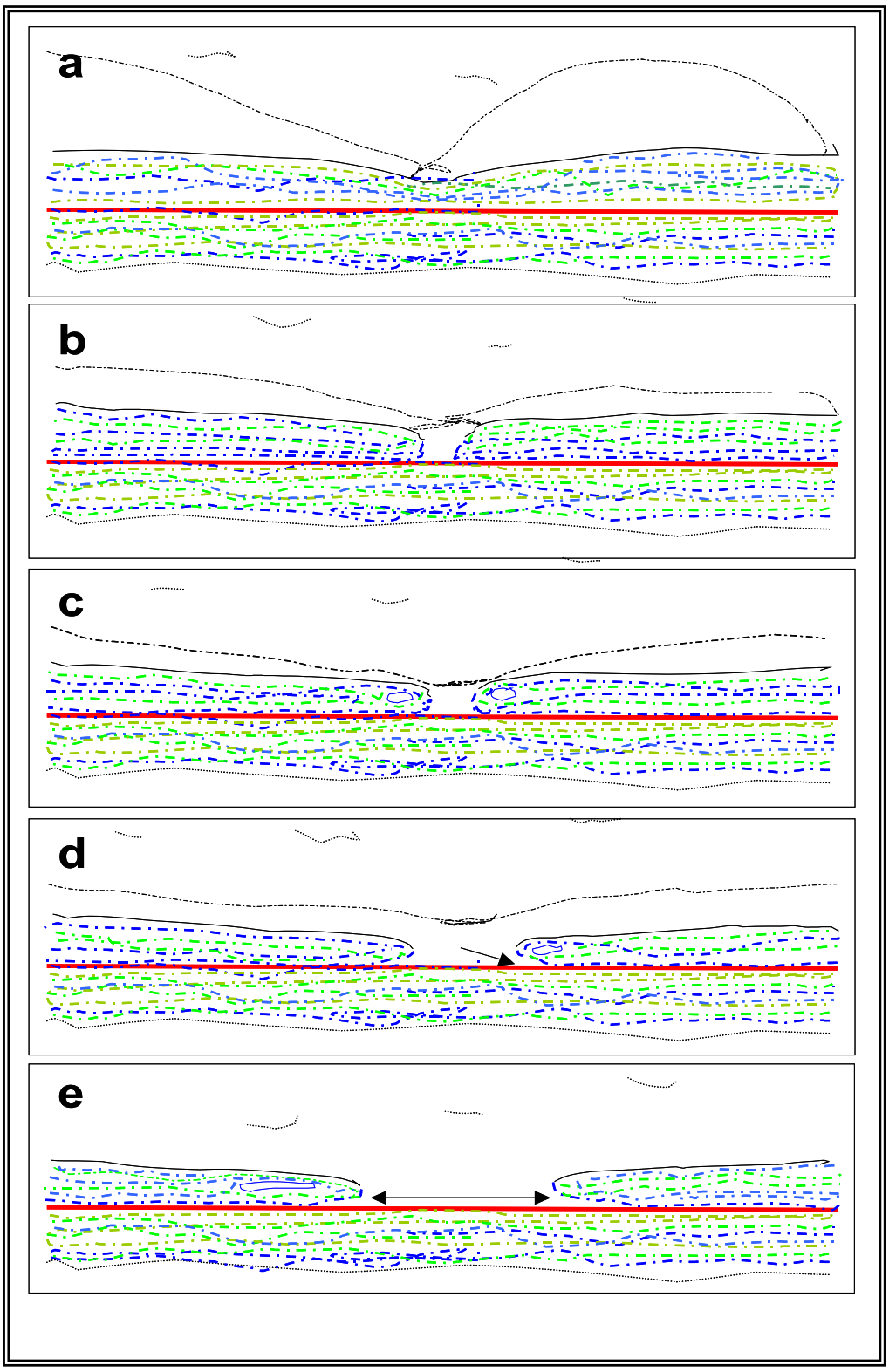

Figure 4 - Schematic evolution of idiopathic FTMH on OCT. Note a central foveal detachment and a high reflectance interface in the preretinal plane which is though to represent the posterior vitreous cortex, partially detached at the macula but remaining tethered to the central fovea **(a)**. Later the stage 1b is noted on OCT, with a more extensive foveal detachment **(b)**. After the onset of the symptoms, the OCT confirms the presence of a pericentric full thickness break and the early formation of an operculum **(c)**. Stage 3 FTMH, with vitreomacular separation and an operculum suspended on the posterior vitreous face. A prominent sub-retinal fluid cuff is visible **(d)** (arrow). Stage 4 FTMH, with extensive sub-retinal fluid cuff (arrow) and the prominent cystoid spaces at the level of the inner plexiform layer at the edge of the hole **(e)**.

Comparable images of a posterior hyaloid partially detached over the curvature of the posterior pole have been shown by ultrassonography by Johnson and associates (Invest Ophthalmol Vis Sci 39, Suppl: S690, 1998) but thanks to its thinner definition, OCT is able to detect earlier stages of incomplete separation of the posterior hyaloid from the macular area. The fact that the profile of the partially detached posterior hyaloid changed from a biconvex shape to a cord or a dome extending over the posterior pole supports the possibility that the hyaloid is tightened by anteroposterior or oblique forces. During the process of macular hole formation, these anteroposterior tractional forces may be transmitted to the foveal floor through the oblique focal attachment of the posterior hyaloid (Chauhan et al, 2000). Controversies have been reported in the literature concerning the nature of pre-hole stages. These stages were defined as macular cyst (McDonnel et al, 1982), involutional macular thinning (Morgan et al, 1988) or stage 1 impending macular hole (Gass et al, 1988). The existence of a foveal cyst as the first step in macular hole formation was at one time widely accepted (McDonnell et al, 1982; Morgan et al, 1988; Frangieh et al, 1981; Kornzweig et al, 1950) but was later rejected on the basis of biomicroscopy findings (Johnson et al, 1988). Subsequently, however Kiryu and colleagues (1995) using laser slit biomicroscopy, were able to show the presence of intra-foveal cysts. Since then images of foveolar cysts has been showed by retinal thickness analyser (Asrani et al, 1998; Folk et al, 1998), scanning laser ophthalmoscope (Kishi et al, 1995) and OCT (Hee et al, 1995).

The OCT findings suggest that FTMH develop in a situation where there is failure of normal age related separation of the vitreous cortex from the posterior pole as a result of an abnormally tenacious attachment to the fovea. The residual attachment at the foveal centre, in the trampoline configuration, may act as a focal point where mechanical forces are transmitted from the vitreous to the foveolar surface leading to foveal traction. Clearly, further studies are required to confirm these findings and to determine the validity of these concepts; in particular, detailed natural history studies correlating the clinical progression of FTMH with the OCT findings. Clinicopathological correlation between biomicroscopic and OCT findings and the cone density in opercula may shed further light on the precise mechanisms leading to hole formation. Such studies will help to define the risk characteristics for progression in early lesions and risk factors for visual outcome after surgery.

Haouchine and colleagues (Haouchine et al, 2001) recently reported a prospective series of 22 eyes exhibiting a foveal pseudocyst on biomicroscopy that were examined and followed with OCT scanning. All 22 eyes were found to have perifoveal vitreous detachment with persistent vitreofoveolar adhesion. They concluded that foveal pseudocysts are a specific entity occurring either as a primary ocular involvement or in the fellow eye of an eye with a macular hole. Foveal pseudocysts are the first step of full thickness macular hole (FTMH) formation, but they also evolve into a lamellar hole, may persist unchanged for months or may resolve completely. Foveal pseudocyst formation may be the result of incomplete separation of the vitreous cortex at the foveal centre and the particular structure of foveal Müller cells.

C-3. Macular Hole Classification

C-3.1. The Original Glass Classification (Gass et al, 1988; Johnson et al, 1988)

Stage 1 – Foveolar detachment or "impending hole"

Is characterised by progressive loss of the foveal depression, associated with the appearance of initially a yellow spot of approximately 100-150 μm at the fovea (stage 1a), later enlarging to a yellow ring (stage 1b).

These changes were attributed to contraction of the prefoveal vitreous cortex with centripetally directed tangential force, leading to anterior displacement of the foveolar retina and greater visibility of the centrifugally displaced xanthophylin. In stage 1 lesions, the posterior vitreous cortex is attached and the retina surface is intact without any evidence of hole or subretinal fluid cuff.

The visual acuity at this stage is often good (20/20-20/60) (Gass et al, 1988; Johnson et al, 1988; Kokame et al, 1995) and blurring and metamorphopsia are the primary complaints. These symptoms are the rule not the exception when a patient presents with involvement of the fellow eye but may be unnoticed in the initial eye, particularly if this is the non-dominant eye.

Stage 2 – Early FTMH

Further traction results in the formation of a foveal hole or dehiscence associated with a cuff of subretinal fluid. At this stage, the posterior cortical vitreous is still attached at the fovea and, in some cases; radial tractional striae may be visible around the edges of the hole at the level of the inner retina and internal limiting membrane. In addition, intraretinal cystoid spaces appear around the edges of the hole.

Two configurations may occur at stage 2:

Centric: the full thickness tear begins at the centre of the fovea (umbo dehiscence) and expands in a symmetric fashion.

Pericentric: a full thickness tear arises at an eccentric position in the fovea and extends in a "can opener" fashion to form a crescent hole progressing to a "horseshoe" shaped hole and eventually, when the can opener is complete, to a round hole with a fully detached operculum of tissue suspended on the posterior vitreous cortex in the prefoveal plane. This configuration is typical in the majority of sage 2 FTMH (80-90%).

The visual acuity usually deteriorates to a level of 20/40-20/100 (Gass et al, 1988; Johnson et al, 1988) and metamorphopsia increases.

Stage 3 – Fully developed FTMH without posterior vitreous detachment

With enlargement of the hole, vitreofoveal separation occurs at the macula. The localised separation of the vitreous cortex is usually only detected because of the presence of the operculum suspended on it lying a short distance (between 100 and 500 μm) anterior to the retinal plane. The fully detached operculum, particularly in cases with more widespread vitreofoveal separation, becomes mobile and this may be detected with eye movements. Gass attributed further enlargement of the hole to passive forces resulting from elastic tissue forces within the retina. Stage 3 holes usually measure more than

350 μm and may occasionally reach a diameter of greater than 500 μm. In addition, as the hole enlarges, discrete white deposits appear on the surface of the retinal pigment epithelium (RPE) at the base of the hole, which represent nodular proliferations of RPE cells. In more long standing cases, a pigmented demarcation line at the level of the RPE may be seen surrounding the cuff of fluid and diminution of the striae and cystic changes may occur.

Stage 3 holes are usually associated with acuity levels of 20/60-20/200 (Gass et al, 1988; Johnson et al, 1988), prominent metamorphopsias, and on occasion a pericentral positive scotoma.

Stage 4-FTMH with posterior vitreous detachment

In about 20% of cases, a full posterior vitreous detachment (PVD) occurs with the presence of a Weiss ring. The operculum can often be seen, on the mobile posterior vitreous face in the vitreous cavity.

In the majority of cases, little or no further enlargement of the hole occurs thereafter with little significant functional deterioration.

C-3.2. The Revised Glass Classification

In the light of the results and in his recent reappraised theory (Gass, 1995), Gass suggested that the majority of FTMH arise from an umbo dehiscence without loss of foveal tissue. He hypothesised that the stage 1b lesion is caused by centrifugal displacement of xanthophylls associated with foveolar detachment, and following a retinal dehiscence at the umbo, by passive enlargement of the “occult” hole beneath the semi opaque, contracted vitreous cortex bridging the edges of the hole. Thus, at this stage, biomicroscopy cannot differentiate between an impending hole and an occult hole. Subsequently, a tear occurs in the contracted vitreous cortex overlying the occult hole, over the edge (pericentric/eccentric stage 2) of the occult hole and the hole becomes visible on biomicroscopy as a stage 2 lesion. Enlargement of the eccentric tear in a can opener fashion occurs to form a “pseudo-operculum” composed of vitreous collagen, glial tissue but no photoreceptors. In some cases, rapid and complete vitreofoveal separation occurs and the umbo dehiscence becomes immediately visible on biomicroscopy as a centric stage 2 lesion. With vitreofoveal separation, the FTMH evolves into a stage 3 and eventually a stage 4 when a full PVD develops.

In conclusion (Table I), impending macular holes (stage 1a) is characterized by flattening of the umbo (loss of foveal depression) and a central yellow spot in the macula. In stage 1b there is a yellow ring with loss of the foveal depression. Initially it was suggested that stages 1A and stages 1B macular holes represented progressive foveolar serous detachments without vitreofoveolar separation. Recent ocular coherence tomography (OCT) data suggest that perifoveal posterior hyaloid separation with persistent adherence of the posterior hyaloid to the foveal centre is the first event in macular hole formation (Hee et al, 1995; Gaudric 1; Haouchine et al, 2001; Mori et al, 2000). This results in an intraretinal split that progresses into intraretinal cystic changes corresponding to the clinical features of stage 1 macular hole. Stage 2 is characterized clinically by a small retinal defect (hole) inside the yellow ring. Ocular coherence tomography demonstrates stage 2 to be a complete, full-thickness retinal defect. In stage 3 macular holes, a larger (≥ 400 micron) hole is apparent with a rim of elevated

retina and complete separation of the posterior hyaloid from the macula. An operculum on the posterior hyaloid may or may not be clinically apparent, but is usually seen on OCT. Stage 4 macular hole is present when the posterior hyaloid separates from the optic disc.

Table I. Macular hole Gass classification.

	Characteristics	Appearance	Type	Posterior Hyaloid	Visual Acuity	Primary Complaints
Stage 1	Loss of foveal depression	Yellow spot (**100-150 μm**) at the fovea Yellow ring at the fovea	**a** **b**	Hyaloid attached to retinal surface	20 / 20 to 20 / 60	Blurring and metamorphopsias
Stage 2	Foveal hole or dehiscence with cuff of subretinal fluid	Tear at the centre of the fovea Tear eccentric in the fovea	**Centric** (10 – 20 %) **Pericentric** (80 – 90 %)	Attached to retinal surface	20 / 40 to 20 / 100	Metamorphopsias increases
Stage 3	Enlargement of the hole	Vitreofoveal separation at the macula (**350 – 500 μm**)	White deposits on RPE (**recent**) Pigmented line on RPE (**old**)	Operculum suspended or lying a short distance of the retina	20 / 60 to 20 / 200	Prominent metamorphopsias and pericentral scotoma
Stage 4	Operculum on the mobile posterior vitreous/hole	No enlargement or sub-retinal fluid	-	PVD with Weiss ring	Same or worse than 3	Same as stage 3

C-4. Natural History of Idiopathic Macular Holes

The progression from stage 1 to stage 2 and from stage 2 to stage 3 usually takes several weeks or months, although in some cases the lesion may remain static without progression at any of the stages described. In most patients it is difficult to establish with any certainty the exact duration of the lesion, as many stage 1 and some stage 2 lesions are asymptomatic, particularly if occurring in the first eye of the patient. In fact, very few lesions present at stage 1 and most patients only experience symptoms and attend for examination when a full thickness defect has become established at the fovea. Most patients with a first eye lesion usually only notice symptoms by coincidence, either after happening to cover the normal fellow eye, or when attending for routine refraction. In contrast, patients with a second eye lesion will often give an accurate account of the onset of symptoms and the duration of the lesion may be determined with grater certainty (Ezra, 2001).

Although numerous studies have reported outcomes after surgical management of macular holes, few studies have addressed the clinical course of unoperated macular holes. The natural history of

macular holes from the “observation” groups was reported in the Vitrectomy for the Prevention of Macular Hole Study Group (De Bustros et al, 1994) and the Vitrectomy for Macular Hole Study Group publications (Kim et al, 1995; Kim et al, 1996). In the former study, 40% of the stage 1 (impending macular hole) eyes randomised to observation progressed to full-thickness macular holes (stage 2-4) during the 17 months of follow-up (De Bustros et al, 1994); in the latter study, only 20% of stage 2 macular holes randomised to vitrectomy progressed to stages 3 and 4 with 12 months of follow-up, compared with 74% in the observation group ($p < 0.006$) (Kim et al, 1995; Kim et al, 1996). Follow-up of unoperated holes demonstrated an increase in the diameter of the hole and declining visual acuity. In Casuso and associates study of unoperated macular holes (5 years median follow-up, 65 eyes, retrospective study, stage 2-4 macular holes) (Casuso et al, 2001), unoperated stage 2 macular holes increased in size and progressed in stage during the follow-up interval. In a retrospective study of 154 eyes with macular holes (stage 1-4) with a median follow-up of 3 years, Hikichi and associates (Hikichi et al, 1995) reported that hole enlargement and vision loss of two or more Snellen acuity lines occurred more commonly among patients with stage 2 holes than among patients with stages 3 and 4 macular holes. The authors concluded that surgery must be beneficial for those patients with stage 2 macular holes because such patients have more vision to loose. In 1996, a study concentrating on the incidence of bilaterality of macular holes by Lewis and colleagues (Lewis et al, 1996) reported that during 36 months of follow-up, all eyes with stage 2 macular holes had visual acuities better than 20/200. For those eyes with stage 3 or 4 macular holes at presentation, 79% had vision of 20/200 or worse with 36 months of follow-up. The Casuso`s study (Casuso et al, 2001) provides further evidence that progressive visual loss usually occurs among patients with early unoperated macular holes and that visual acuity generally stabilizes at the 20/200 to 20/400 level.

In a prospective study of 122 patients with idiopathic macular holes, Chew and associates (Chew et al, 1999) reported that 44.8% of eyes had a decrease in visual acuity of two or more Snellen acuity lines at various follow-up intervals. Moreover, an inversely proportional association was reported between best-corrected visual acuity at baseline and diameter of macular hole, with smaller holes having better visual acuity at presentation. There was no significant association between baseline macular hole diameter and visual acuity years later. Similarly, Casuso (Casuso et al, 2001) demonstrated that macular hole stage at baseline was not significantly associated with visual acuity at 5 years or at final follow-up. Presenting visual acuity, regardless of macular hole size, was significantly associated with visual acuity at 5 years and at final follow-up (Casuso et al, 2001). In this study, long-term follow-up of unoperated macular holes demonstrates progression in hole size and stage, vision loss which generally stabilizes at the 20/200 to 20/400 level, a redistribution and reduced number of yellow nodular opacities at the level of the retinal pigment epithelium, and the development of retinal pigment epithelial atrophy surrounding the macular hole, resulting in a “bull’s-eye” macular appearance.

Johnson and Gass (Johnson et al, 1988) and Kokame and associates (Kokame et al, 1995) observed that approximately 66% of stage 1 lesions progressed to FTMH, somewhat higher estimates compared with those of 37% by Akiba (Akiba et al, 1990) and 10.5% by Guyer and colleagues (Guyer et al, 1992).

More recently, Kim and associates (Kim et al, 1995) reported the results of a randomised trial, which included an observation arm for stages 2 holes. They found a 55% progression rate to stage 3 for centric stage 2 holes, compared with 100% for pericentric (can opener) holes, with an overall progression rate of all stage 2 holes to either stage 3 or 4 of 74%.

It is well recognised, that 30-50% of stage 1a and 1b lesions will arrest or resolve spontaneously often with resolution symptoms in some eyes (Gass et al, 1988; Johnson et al, 1988; Kokame et al, 1995; De Bustros et al, 1994). In such cases, arrest usually occur following vitreofoveal separation, and a good visual acuity is a favourable prognostic indicator (Kokame et al, 1995).

Gass has described the clinical features of arrested lesions (Gass et al, 1988). The fovea may have an entirely normal contour or may demonstrate a residual small inner lamellar defect or larger inner lamellar defect often with an operculum on the separated vitreous face. In such cases, patients may notice the operculum, as a small dark mobile object in the visual axis but the visual acuity is usually normal or near normal. In some patients, the operculum may cast a shadow onto the fovea, during slit lamp examination, which may be mistaken for a FTMH.

Spontaneous closure may also occur in a stage 2 or 3 lesions, although this is relatively rare, occurring in less than 10% of cases (Gass et al, 1988; Johnson et al, 1988; Kim et al, 1995; Kakehashi et al, 1995). Again this is usually associated with vitreofoveal separation in stage 2 holes and further vitreofoveal separation or a full PVD in stages 3 holes. The closed hole may demonstrate a virtually normal foveal reflex or a lamellar defect as described above and the visual acuity may recover to the 20/20-20/60 level. In the majority of cases, vitreofoveal separation with an operculum is seen and only a minority will develop a full PVD.

C-5. Incidence of Macular Holes in Fellow Eyes

Although FTMH is an important cause of central visual loss with a prevalence of 3.3 per 1000 (Ezra, 2001), the majority of patients have unilateral involvement at presentation and are able to continue with good visual function from the fellow eye. Thus, the risk to the fellow eye is an important factor for both patients and clinicians in determining whether surgical treatment of the first eye is pursued. In particular, the risks of surgery, anaesthesia, and the postoperative posturing regimens have to be considered very carefully in older patients, especially if the risk to the normal fellow eye is low. In assessing the risk to a fellow eye without a hole, two important factors have to be considered: (1) the presence of a predisposing foveal lesion such as an impending hole indicates a higher risk of progression to FTMH in the region of 40-60%; and (2) the presence of a PVD, as indicated by a Weiss ring, is associated with an extremely low risk of progression to FTMH of less than 1%.

Although a number of studies, mostly retrospective, have reported on the incidence of FTMH in fellow eyes, ranging between 0% and 29% (Ezra, 2001), the data have been difficult to interpret as some studies have not differentiated between normal fellow eyes and fellow eyes with possible predisposing lesions such as macular cysts and impending holes, while others have not differentiated between normal fellow eyes with and without a PVD.

In a retrospective study of 69 patients, Aaberg and associates (1970) found that 7% of patients had bilateral FTMH at presentation, while a further 11% developed a hole in the fellow eye during a mean follow up of 19 months, although in this study, no differentiation was made between fellow eyes with a predisposing foveal lesion and fellow eyes with a normal fovea. In another retrospective study, Bronstein and colleagues (1981) reported a 7% incidence of bilaterality on presentation and a 12% incidence of FTMH in initially normal fellow eyes over a mean follow up of 57 months, while in contrast McDonnell and associates (1982), in a similar analysis, found a 2% bilaterality at presentation, with a 0% progression in initially normal fellow eyes at a mean follow up of 27 months. Neither of these studies, however, differentiated between initially normal fellow eyes with and without a PVD at presentation. Trempe and colleagues (1986), in a retrospective study of 49 eyes, found a bilaterality of 3% at presentation, and on subsequent mean follow up of 47 months, 28% of fellow eyes without a PVD at presentation developed a FTMH compared with 0% of fellow eyes with a PVD at presentation. However, this study did not differentiate between fellow eyes with and without predisposing foveal lesions and it is likely that the higher progression rate in fellow eyes was biased by the inclusion of fellow eyes with impending holes at presentation.

In a prospective 5-year cohort study of 114 normal fellow eyes without a PVD, conducted at Moorfields Eye Hospital, Ezra and colleagues (1998) found an incidence of 7.5% at 18 months and 15.6% at 5 years, as determined by Kaplan-Meier analysis. The Eye Disease Case-Control Study group reported a cohort of 198 patients with macular holes and normal fellow eyes examined at baseline, of which 122 (71%) were available for follow-up, with a rate of fellow eye involvement of 4.3% at 3 years and 6.5% at 5 years. No details were available on the presence or absence of a PVD in fellow eyes at baseline.

Thus, from the data available to date, it appears that in patients with a unilateral FTMH, the risk to a normal fellow eye without a PVD is in the region of 10-20%, while the risk is extremely small; probably less than 1% if a PVD is present. The overall risk to a normal fellow eye is in the region of 5-10% over 5 years. The risk to a fellow eye with an impending hole is much higher and is in the region of about 40-60% (Ezra, 2001).

C-6. Examination and Diagnosis

Although impending macular holes and FTMH may be confused with a number of other foveal and macular lesions, careful slit lamp biomicroscopy is usually sufficient to establish the diagnosis in the majority of cases. In particular, fundus contact lens examination should be performed in all cases where doubt exists. The Watzke-Allen test (Watzke et al, 1969) is extremely useful in differentiating FTMHs from other lesions. In this test, a very narrow slit beam is projected with a 90 or 78 dioptre lens onto the fovea and the patient is asked to describe the line. Most patients with a stage 3 FTMH report a break in the central portion of the line (Watzke-Allen positive), whereas those with small holes (with a relatively intact foveal photoreceptor population) or other lesions associated with metamorphopsia may report “thinning” (Watzke-Allen negative). A more reliable test may be performed, by projecting a

50 μm HeNe laser spot onto the centre of the suspected FTMH; the patient with a FTMH will not perceive the spot (Gass et al, 1988; Johnson et al, 1988).

Fundus fluorescein angiography is rarely necessary in FTMH and in the vast majority; careful examination may establish the diagnosis and stage. The angiographic findings at the various stages of macular hole development have been described (Aaberg, 1970; Gass et al, 1988; Gass, 1995; Gass, 1970; Johnson et al, 1988; Kim et al, 1995; Freeman et al, 1997; Kim et al, 1996). In impending FTMH, early faint central focal hyper fluorescence is seen at the fovea in some cases, while in others a normal angiogram may occur. In stages 2 holes, the central early hyper fluorescence is usually more pronounced and fades during the later stages of the run; however, in some cases no hyper fluorescence is observed. Thus, fluorescein angiography (FA) cannot differentiate with any certainty between stage 1 and 2 lesions and clinical examination is often more reliable. In stage 3 and 4 lesions, the hyper fluorescence is more obvious and is frequently associated with changes at the RPE level, which may also be seen biomicroscopically. In these lesions, the cuff of subretinal fluid surrounding the hole may appear either as a hyper fluorescent or hypo fluorescent ring around the central area of hyper fluorescence.

Other techniques such as confocal scanning laser ophthalmoscopy, microperimetry, auto-fluorescence and retinal thickness analysis, have also been used to assess macular holes (Weinberger et al, 1995; Hudson et al, 1997; Beausencourt et al, 1997; Guez et al, 1998; Ruckmann et al, 1998; Asrani et al, 1998).

Optical coherence tomography (OCT), a relatively new technique, has also been used to allow detailed cross sectional examination of macular holes and may be effective in distinguishing them from other lesions where doubt exists (Hee et al, 1995).

C-6.1. Optical Coherence Tomography of Macular Holes

Recent advances in the surgical treatment of idiopathic and secondary macular holes have increased the chances of recovering or preventing vision loss in patients with this disease. Lesions that ophthalmoscopically resemble various stages of macular hole development, however, can be relatively common. Optical coherence tomography (OCT) is a new diagnostic imaging technique for high-resolution, cross-sectional imaging of the anterior and posterior segments of the eye (Huang et al, 1991; Swanson et al, 1993). This technique is analogous to ultrasound B-scan except that it uses light rather than sound to obtain much higher image resolution (approximately 10 μm) in the retina with a non-contact measurement (Hee et al, 1995).

The ability to differentiate a FTMH from a macular pseudohole, partial-thickness hole, or a macular cyst is important in defining appropriate treatment. The high-resolution tomographic images provided by OCT could directly identify full-thickness holes by their complete loss of retinal tissue in the fovea (Hee et al, 1995). In contrast, macular pseudo-holes and partial-thickness holes showed an altered foveal pit contour with an intact outer neurosensory retina. Macular cysts appeared as localized, non-reflective cavities within the retina on OCT examination. The distinction between a macular pseudo-

hole and a lamellar hole with OCT was more difficult. An epimacular membrane only appeared distinct from the retinal nerve fibber layer on the OCT as a thin, moderately reflective band anterior to the retina (Hee et al, 1995).

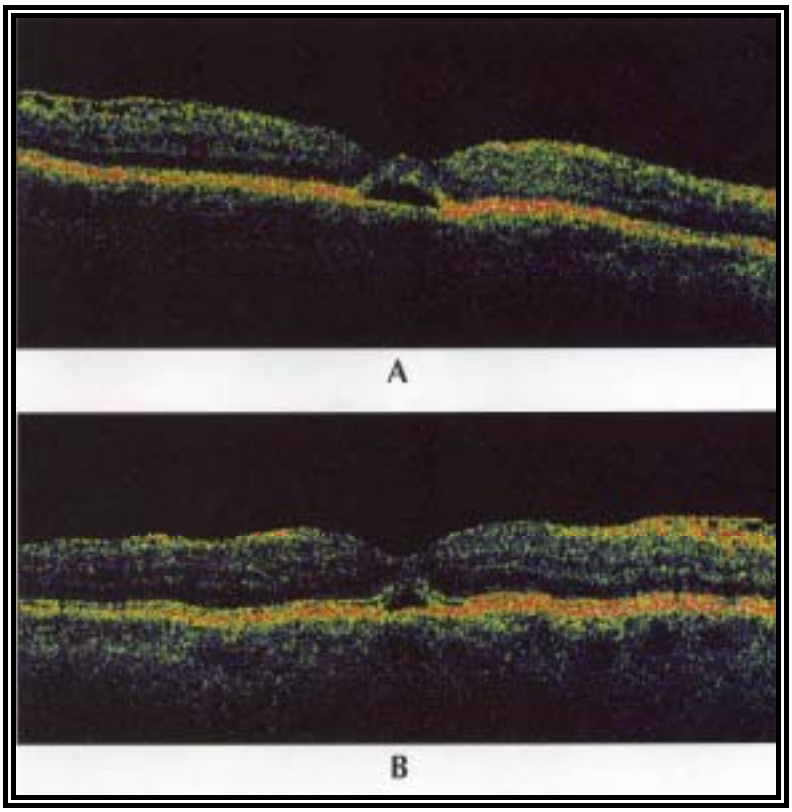

Figure 5 – OCT in MH. OCT reveals separation of the retina from the RPE layer in the fovea, defining stage IA macular hole (A). The foveal contour is still present, 3 months later (B).

Accurate staging of macular holes also may be important for evaluating and planning possible surgical interventions. Stage 1 impending holes were recognizable on OCT examination by their reduced or absent foveal pit, and the presence of a minimally reflective space just beneath or within the fovea (Figure 5). In stage 1, some authors (Kishi et al, 1995), believe that foveal cysts are the initial stage of macular hole formation (Figure 5 B).

The anterior wall of the cyst becomes an operculum. Some reports using laser slit beams have described cystic changes at the fovea in eyes with the first stage of a macular hole (Asrani et al, 1998; Folk et al, 1998). Macular holes start as a retinal split or foveal cyst in most cases. The anterior wall of the cyst serves as a flap in stage 2 and an operculum in stage 3 holes. Radiating striae correspond to retinal splits or cysts and presumably represent an elevation of Henle fibber. In a few macular holes, foveal detachment is the initial change. The detached retina thins and eventually develops a hole. In both courses, anterior traction of slightly detached vitreous cortex appears to be a major contributing factor to macular hole formation (Kishi et al, 2000) (Figure 6 A).

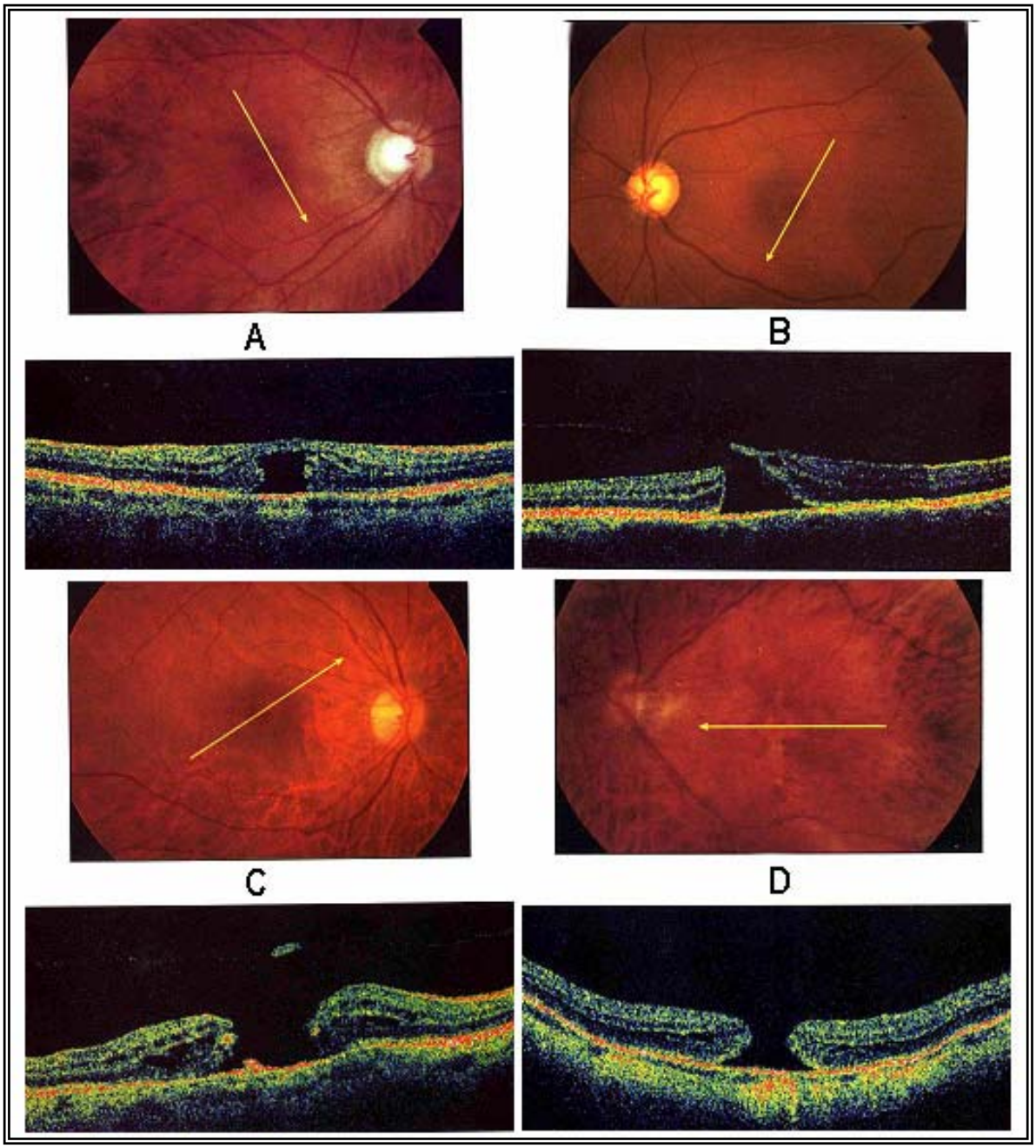

Figure 6 – OCT and MH diagnosis and classification. Stage 1 b macular hole (A), with elevation of the foveal retina to the level of the vitreoretinal interface. An operculum can be seen, in stage 2 (B). Stage 3 macular hole (C), with a fully developed hole with an operculum. Full-thickness macular hole (D) stage 4, with PVD.

OCT images of stage 2 macular holes showed a partial break in the surface of the retina with a small full-thickness loss of retinal tissue (Figure 6 B). OCT revealed optically empty space at the fovea, which was mostly or partially covered by a flap-like tissue extending from the perifoveal retina (Kishi et al, 2000) and the vitreous cortex, which was attached to the flap but slightly detached in the perifoveal area in the majority of the cases. OCT obtained through stage 3 holes demonstrated a fully developed hole, with a full-thickness retinal defect at the fovea (Figure 6 C). An operculum was sometimes visible and was completely separated from the edges of the hole, and in some cases there are cystic

changes in the perifoveal retina. The degree of vitreofoveal separation could be assessed by the distance of the operculum from the surface of the retina, or in some cases by direct observation of the posterior hyaloid membrane on the OCT scans. The OCT images also showed a variable range of surrounding retinal detachment and subretinal fluid accumulation. A complete separation of the posterior vitreous from the optic disc and macula was seen in the OCT of stage 4 macular holes (Figure 6 D). An arrested macular hole was recognized in an OCT cross-section by the presence of a pseudo-operculum suspended above a normal-appearing foveal pit.

In summary, OCT is a new technique for diagnosing and quantitatively monitoring macular hole development. On OCT, macular holes from ophthalmoscopically similar-appearing lesions can be distinguished effectively and the stages of hole development can be tracked. The micron-scale resolution of OCT is useful for quantitatively assessing hole diameter and the amount of retinal thickening and edema surrounding a hole, allowing sensitive monitoring of hole progression or recovery after treatment. Finally, OCT appears potentially useful in evaluating the vitreoretinal interface, an important parameter in the development of macular holes.

In conclusion, the OCT is useful in macular holes approach for: a) the differential diagnosis of stage 1 holes from other subtle spots at the macula, and also can unequivocally classify stage 2-4 holes; recent evidence from OCT has shed light on the pathogenesis and natural history of macular holes; b) examination of the fellow eye may be of prognostic value; c) preoperatively, an OCT scan may show the presence of a coexisting epiretinal membrane (ERM), which might need to be peeled during vitrectomy; d) postoperatively, OCT will clearly demonstrate the extent of hole closure.

C-7. Surgical Treatment

C-7.1. Macular Hole Surgery

The aim of vitreoretinal surgery for traction maculopathies is the thorough removal of epiretinal collagen fibbers and fibro cellular membranes to relieve tangential and anterior-posterior traction. In theory, there are two different planes of dissection: (1) directly on the inner, vitreal surface of the ILM, which can be experimentally achieved by enzymatic induction of PVD and (2) between the retinal surface of the ILM and the foot-plates of Müller cells, which can be achieved only surgically; in theory, epiretinal cellular layers could be removed along with the ILM. In other words, instead of removing tractional forces in front of the retina as achieved by enzymatic PVD, with ILM peeling the surgeon eliminates traction by removing the retinal surface itself.

Nevertheless, the clinical relevance of the ILM for macular surgery was not understood before the 1980s, when histopathological examinations of removed epiretinal membranes disclosed that fragments of the ILM were a common feature of these membranes (Kampik et al, 1980; Kampik et al, 1981). No functional deficits were noted in these patients. However, this unintended ILM removal

could not be transferred into intentional clinical practice at that time. The question of intentional ILM removal emerged in the late 1980s, when Donald Gass presented his classification of macular holes (Gass, 1988), which contributed significantly to our understanding of the pathogenesis of this disease. This report coincided with the first report on successful vitrectomy for macular hole closure by Kelly and Wendel, in 1991. Morris and Kuhn, first described intentional ILM removal, in patients with Terson syndrome (Morris et al, 1994). In cases of sub-ILM haemorrhages, ILM peeling led to excellent functional results even in the long term; as a consequence, ILM peeling was then suggested as an approach to treat other traction maculopathies.

Recent innovations in macular imaging and surgery have provided important new information concerning the pathogenesis and treatment of idiopathic macular hole. New imaging data suggest that localized perifoveal vitreous detachment (an early stage of age-related posterior vitreous detachment) is the primary pathogenic event in idiopathic macular hole formation. Detachment of the posterior hyaloid from the pericentral retina exerts anterior traction on the foveola and localizes into the foveola the dynamic vitreous traction associated with ocular rotations. OCT has clarified the path anatomy of early macular hole stages, beginning with a foveal pseudocyst (stage 1A) and typically followed by disruption of the outer retina (stage 1B) before progressing to a full-thickness dehiscence (stage 2). The treatment of macular holes continues to evolve as modifications to the standard surgical procedure. These innovations include the use of adjuvant, peeling of the internal limiting membrane, vital staining of the ILM and variations in the length and tamponade of postoperative period.

In all studies retrieved over 400 citations (Benson et al, 2001), the definition of surgical success varied; some studies considered flattening of the edges of the hole to be a surgical success while others required closure of the edges of the hole. Most studies did not standardize follow-up period or adjust for time differences in the analysis of functional outcomes. When visual acuity and hole closure are assessed at varying intervals, it is difficult to attribute these results to the surgery, because patients were assessed at different stages of recovery or disease progression. Many studies did not control for prognostic factors such as duration of symptoms, size, and presence of epiretinal membrane (Benson et al, 2001). Further, some series included operations on stage 2 holes, which may have a better prognosis.

C-7.2. Visual Loss Reversibility and Complications, from Macular Hole Surgery

Freeman and associates compared the value of surgery versus observation for stage 3 or 4 macular hole (Freeman et al, 1997) In the surgery group, 36 of the 52 (69 %) stage 3 or 4 holes were closed compared with only 2 of 56 (4 %) eyes in the observation group ($p < 0.001$). Statistically the surgically treated eyes had significantly better visual acuity at 6 months, as reflected by better ETDRS chart visual acuity (20/15 vs. 20/166, $p < 0.004$) and Bailey-Lovie word reading test scores (20/155 vs. 20/166, $p < 0.01$). No significant differences were found between the two groups for word reading speed scores. After adjusting for baseline visual acuity and hole duration and size, the benefit of surgery persisted for word reading ($p = 0.02$) and marginally for ETDRS visual acuity ($p = 0.05$). A final

visual acuity of 20/26 or better was achieved in 11 eyes in the surgery group as opposed to 2 eyes in the observation group. A clear benefit in closure rate and final visual acuity was shown, even though the mean hole duration was 18.8 months in the surgery group and 28.6 in the observation group. The benefits of surgery for stage 3 or stage 4 macular hole were demonstrated.

The outcomes of surgery for FTMH in uncontrolled studies are presented in Table II (Brooks, 2000, Kang et al, 2000, Margherio et al, 2000; Mester et al, 2000; Park et al, 1999; Olsen et al, 1998; Minihan et al, 1997; Gaudric et al, 1997; Tornambe et al, 1997; Smiddy et al, 1997; Polk et al, 1996; Lansing et al, 1993). The more favourable anatomic and visual outcomes noted in these series may be attributed to including patients with macular holes of shorter duration than those of patients included in the randomised trials. In addition, the results may reflect advances in surgical technique and experience. Patients report a benefit from macular hole surgery, even though they must undergo surgery, maintain an uncomfortable position for a week or more, and likely to need cataract surgery.

Polk and associates closed 86 % of macular holes with one operation (Polk et al, 1996). Six patients had a reoperation resulting in the closure of an additional macular hole. Of the 71 eyes, 49 % (35) had a final vision of 20/40 or better. In patients with 20/40 or better in the fellow eye, the operated eye became the better eye in 9/48 (19 %); in patients with 20/50 or worse in the fellow eye, the operated eye became the better eye in 70 % of cases, bilateral visual function improved by one level in 39 %, and the average visual impairment decreased from 52 to 35 % (Polk et al, 1996).

In 25 of the 30 eyes (83 %) that Pearce evaluated, the macular hole was closed (Pearce et al, 1998). Half of the patients had two more lines of improvement and 27% had visual acuity of 20/40 or better. In terms of patient satisfaction, 53 % said that they could read a newspaper better, 70 % could see faces better, and 57 % could read bus numbers better.

In addition to the cost of the surgery and the difficulty of maintaining a facedown postoperatively, there are ocular complications. Acute complications include retinal tears in about 3 % of operations (Paques et al, 1999; Polk et al, 1996; Park et al, 1999; Minihan et al, 1997; Minihan et al, 1997; Sjaarda et al, 1995; Park et al, 1995) and occasional cases of endophthalmitis (Freeman et al, 1997; Park et al, 1995; Banker et al, 1997). Long-term complications include nuclear sclerotic cataract in the vast majority of patients (Polk et al, 1996; Thompson et al, 1995; Leonard et al, 1997) and retinal detachment in 1 % to 3 % (Paques et al, 1999; Park et al, 1999; Minihan et al, 1997; Sjaarda et al, 1995; Park et al, 1995; Banker et al, 1997; Tornambe et al, 1997; Olsen et al, 1998). In addition, the repaired macular hole may reopen in 2 % to 10 % of cases (Park et al, 1995; Banker et al, 1997; Duker et al, 1994; Paques et al, 2000; Chrstmas et al, 1998). Finally, in assessing the value of macular hole surgery, it is important to consider that a patient who has a FTMH in one eye has about 15 % risk of developing a FTMH in the fellow eye (Ezra et al, 1998; Lewis et al, 1996).

Table II. Outcomes of surgery studied in uncontrolled studies.

Study (year)	Modifications to Surgery	Anatomic Success	Minimum Standard of Success	Minimum Follow-up (months)	> 20/40	> 20/50	> 20/60	Lines of Improved Vision
Brooks (2001)	ILM peeling	116/116 (100%)	Closure	6	82/116 (71%)			4.9
Kang (2000)	ILM peeling	51/56 (91%)	Flattening of the edges	3	27/56 (48%)			54% gained 2
Margherio (2000)	Preretinal Tissue/ILM Peeling.	51/59 (85%)	Closure	6		38/59 (64.4%)		
	No peeling	44/48 (92%)				41/48 (84.5%)		
Mester (2000)	ILM peeling	44/46 (96%)	Flat with no subretinal fluid	3				85% gained 2
Park (1999)	ILM peeling	53/58 (91%)	Closure	6		31/58 (58%)		
Olsen (1998)	Fibrinogen only	32/45 (71%)	Flattening of the edges	6				2.8
	Fibrinogen and ILM peeling	23/24 (96%)	Flattening of the edges	6				2.3
Pearce (1998)	APC	25/30 (83%)	Flat with no subretinal fluid	3	8/30 (27%)			
Minihan (1997)	APC	48/50 (96%)	Flat with no subretinal fluid	12	21/50 (42%)		31/50 (62%)	
Gaudric (1997)	APC	72/77 (93%)	Flattening edges	Not Given		52/72 (72%)		
Tornambe (1997)	Face-up positioning	26/33 (79%)	Flattening edges	12		16/33 (48%)		
Smiddy (1997)	ILM peeling	39/43 (91%)	Flattening edges	3	14/33 (33%)			65% gained 3
Polk (1996)	TGFß2	61/71 (86%)	Flattening edges	3				82% gained 2
Lansing (1993)	TGFß2	22/23 (96%)	Flat with no subretinal fluid	12	11/23 (48%)		19/23 (85%)	

(ILM = internal limiting membrane; RPE = retinal pigment epithelium; APC = autologous platelet concentrate; TGFß2 = transforming growth factor ß2).

C-7.3. Stage of the Disease Indicated for Surgery

De Bustros reported a randomised, prospective trial of patients with stage 1 macular hole (de Bustros et al, 1994). All patients had a stage 3 or 4 macular hole in their fellow eye. The patients were randomised to vitrectomy or observation and 97 % were followed for an average of 17 months. In the observation group 14 of 35 eyes (40 %) progressed to stage 3 or 4 macular hole, while in the vitrectomy group 10 of 27 eyes (37 %) progressed (p = 0.81). Postoperatively, 33 % of the surgery group had a visual acuity of 20/80 or worse compared with 20 % of the observation group. The trial was terminated prematurely due to low recruitment, but surgery does not appear to be warranted for stage 1 macular holes.

Kim and colleagues reported a randomised, prospective trial of patients with stage 2 macular hole (Kim et al, 1996). All patients had a full-thickness macular hole in their fellow eye. The patients were randomised to vitrectomy or observation, and approximately 90 % were followed for 12 months. In the observation group 15 of 21 (71 %) eyes progressed to full thickness stage 3 or 4 macular hole. Their mean ETDRS visual acuity was 20/69 deteriorating to 20/80 at 12 months. In the surgery group, only 3 of 15 (20 %) of the eyes progressed to FTMH. The ETDRS visual acuity was stable, 20/60 at baseline and 20/62 at 12 months. Thus, although the observation group had a statistically significant, higher rate of progression to hole formation (p = 0.006), there was no statistically significant difference in the final visual acuity (p = 0.17) between the two groups. However, using the Bailey-Lovie word-reading test, the surgery group had a visual acuity of 20/78, compared with 20/135 in the observation group (p = 0.006). Although the study was a randomised clinical trial, it enrolled only a small number of patients to detect a visual acuity difference between the surgery and observation groups. Since progression from stage 2 macular hole to stage 3 or 4 macular hole is usually associated with visual loss, this study supports surgery for stage 2 macular holes.

C-7.4. Modifications to Surgery

Transforming growth factor ß2 (TGFß2) was investigated with the hope that it would induce glial cells to close the hole (Smiddy et al, 1993; Lansing et al, 1993, Kozy et al, 1996). Successful flattening of the edges of the hole of 91 % to 96 % of cases stimulated the search for adjuvant. They include autologous serum (Banker et al, 1999; Melberg et al, 1996; Liggett et al, 1995; Wells et al, 1996; Kusaka et al, 1997). Thrombin-activated fibrinogen (Olsen et al, 1998), thrombin (Vine et al, 1996), plasmin (Liggett et al, 1995) and autologous platelet concentrate (APC) (Pearce et al, 1998; Minihan et al 1, 1997;Gaudric et al, 1997). Another modification to Kelly and Wendel´s (Kelly et al, 1991) initially reported operative techniques (pars plana vitrectomy with gas-fluid exchange, 1991) are peeling of the internal limiting membrane (ILM) with and without indocyanine green.

Smiddy et al (1993) multicentered, prospective, randomised trial, compared 44 eyes treated with bovine TGFß2 with 44 eyes given placebo. The 3-month results were reported for 100 % of the

patients. In the placebo group, the edges of the hole were flattened in 53 % of the eyes compared with 91 % of the TGFß2 group ($p < 0.001$). The visual results of this study were not reported.
Thompson and associates (1998) multicentered, prospective, randomised trial compared 65 eyes treated with recombinant TGFß2 with 65 eyes given placebo. The 3-months results were reported for 97 % of the TGFß2 and 87 % of the placebo group. In the placebo group the edges of the hole were flattened in 61 % of the eyes compared with78 % of the recombinant TGFß2 group. The difference was not statistically significant ($p = 0.08$). There was also no statistically significant difference in visual acuity results between the two groups. A final visual acuity of 20/40 or better was achieved in 12 % of the placebo group versus 22 % of the TGFß2 group ($p = 0.49$) The mean visual acuity of the placebo group was 20/80 versus 20/80+2 in the TGFß2 group ($p = 0.22$).
Autologous platelet concentrate has been used as an adjuvant because platelets alpha granules contain growth factors (TGFß2 and platelet-derived growth factor), known to promote the wound-healing process. The study reported by Paques was multicentered, prospective, randomised, and double masked (Paques et al, 1999). It compares 53 eyes treated with APC with 57 eyes given placebo. The 6-month results were reported for 91 % of the 110 patients. The hole was closed in 81 % of the placebo group versus 94 % of the APC group ($p = 0.049$). The mean ETDRS visual acuity score was similar in the placebo group and the APC group (56 vs. 60, $p = 0.25$). The inclusion of information, about the proportion of patients with greater than 20/40 visual acuity, might have provided some additional insight.

C-7.4.1. Removal of the Internal Limiting Membrane with or without Indocyanine Green

The removal of the internal limiting membrane (ILM) has been shown to be an effective treatment option in several vitreomacular diseases such as macular holes (Haritoglou et al, 2002), macular pucker, or macular edema (Kampik et al, 2003). As the ILM being part of the vitreomacular interface represents a scaffold for proliferating cells, ILM peeling is currently considered a prophylactic measure against persistent or recurrent macular puckers or macular holes (Haritoglou et al, 2002). Furthermore, the ILM peeling seems to improve postoperative anatomical and functional outcome in macular holes (Brooks et al, 2000). There are only single reports suggesting that ILM peeling correlates with functional deterioration (Sivalingam et al, 1990). A large prospective study revealed small, para central, yet asymptomatic scotomata detected by microperimetry after ILM peeling for macular hole repair (Haritoglou et al, 2002). This observation may indicate a discrete mechanical trauma to the nerve fibber layer associated with ILM peeling (Haritoglou et al, 2001).
However, the ILM is a very delicate and nearly invisible structure, and its removal represents a challenge for the vitreoretinal surgeon. It is not only difficult to grasp the ILM, but also to determine where the ILM has already been stripped off and where additional peeling might be necessary. Therefore, effort was made to develop a technique to visualize the ILM. One recent approach was the introduction of indocyanine green (ICG) for intraocular application during vitreomacular surgery.

A tricarbocyanine dye, indocyanine green (ICG) was initially introduced in 1957. It become popular to record dilution curves to measures cardiac output or organ perfusion and was also used for liver function diagnosis. The principal advantages were the confinement to the vascular compartment by binding to plasma proteins and rapid excretion almost exclusively into the bile. Typically, ICG has a maximum peak of absorption at approximately 800 nm. However, it has been shown that the absorption qualities of ICG show significant variations depending on the solvent medium, for example, plasma or water, and the dye concentration. Another influence on the absorption spectrum results from progressive aggregate formation with increasing concentration (Kampik et al, 2003).

In ophthalmology, the main indication is the intravenous application for the imaging of the choroidal circulation by ICG angiography. The intravenous application of the dye has a long history of safety. Additionally, ICG can be used as a vital dye for donor corneal endothelium and to assist capsulorhesis in eyes with mature cataract. In addition, it was observed that ICG may also stain to some degree the vitreous cortex (especially the anterior vitreous base) and the ILM more intensely around the edges of a retinal hole, but no staining around vessels was seen. Recently, ICG was also suggested for photodynamic therapy at the choriocapillaris layer.

The first description of ICG staining of the ILM was by Vivian Kim at the American Academy of Ophthalmology in 1999 (poster 349). Kadonosono and associates published one of the first reports on ICG-assisted ILM peeling in the year 2000 (Kadonosono et al, 2000). It was, furthermore, demonstrated that ICG selectively stains the ILM, as a staining effect could only be achieved when the vitreous cortex or epiretinal tissue was thoroughly removed (Gandorfer et al, 2001). Additionally, a “negative” staining effect might be useful under special circumstances such as persistent/recurrent holes, where areas of peeled and areas of not removed ILM could be identified during a second surgical approach. Clinic and histopathological evaluations indicated that the ILM is often unintentionally removed at least in part with the epiretinal cellular layer in macular pucker surgery. However, a complete removal of the ILM reduces reproliferation of epiretinal cells and leads to better functional results (Brooks et al, 2000). Therefore, ICG could be used to assist the removal of tissue (ILM) subsequent to the initial peeling of the visible epiretinal fibro cellular layer. Several published studies have emphasized the obvious advantages of the dye in visualization and easier and more complete removal of the ILM. Consequently, dye-assisted vitrectomy has been received with great enthusiasm.

However, there were reports on reduced functional benefit after ICG-assisted vitrectomy for macular holes (Haritoglou et al, 2002). Some authors additionally described alterations of the retinal pigment epithelium possibly attributed to intraoperative ICG dye use (Engelbrecht et al, 2002; Sippy et al, 2001). The incidence of peripheral visual field defects increased to up to 30%, whereas visual field defects appeared less commonly after “conventional” macular hole or macular pucker surgery with ILM peeling (Haritoglou et al, 2002). Of note, no other modifications concerning the operative techniques except the use of ICG were made in these series. Some authors additionally described alterations of the retinal pigment epithelium (Engelbrecht et al, 2002). The histopathologic analysis of tissue harvested during ICG-assisted vitreomacular surgery revealed large fragments of cellular structures resembling the plasma membranes of Müller cells and other cellular debris of unknown

origin adherent to the retinal surface of the ILM (Haritoglou et al, 2002). This finding was interpreted as an alteration of the cleavage plane from the ILM to the innermost retinal layers. Nevertheless, such morphologic alterations alone cannot explain an adverse effect on functional outcome. Consequently, these clinical reports were soon followed by experimental data gained in animal models as well as in vitro evaluations (Sippy et al, 2001; Enaida et al, 2002). These observations suggested toxic effects to the human retina and retinal pigment epithelium. In the light of this, some institutions decided to forego of ICG as an intraoperative dye for the time being (Kampik et al, 2003).

At present, several factors have been suggested as possible underlying path mechanisms. In macular hole surgery, the dye could come into contact with the retinal pigment epithelium through the neurosensory defect resulting in cellular damage. It was shown that ICG auto fluorescence can persist even months after macular hole surgery, which might lead to delayed photochemical damage to the retinal pigment epithelium. However, as adverse effects were also noted after ICG-assisted macular pucker surgery, where such a defect is missing, other mechanisms of action have to be considered, among them hypo-osmolarity of the ICG solution, toxic effects of the dye itself, the dye concentration, or the light absorption qualities of ICG. The photodynamic properties of ICG are known and have been used for therapeutic purposes in others medical fields (Kampik et al, 2003).

Currently, there is no standardized protocol for ICG preparation and intraocular application. Various reports have dissolved and diluted the dye in different solvent media such as balanced salt solution (BSS), BSS plus, glucose, or viscoelastic solution, but also applied ICG in varying concentrations ranging from 0.05% up to 0.5%. Furthermore, it is still not clear whether ICG should be injected into the fluid- or air-filled globe, although it seems likely that higher ICG concentrations are achieved on the retinal surface in the air-filled eye. In contrast, the contact between ICG and the retina is not the limited to the posterior pole when the dye is applied into the fluid-filled eye. Moreover, it is not known how long ICG should incubate within the vitreous cavity because sufficient staining can be achieved even after few seconds.

Additionally, the use of the intraocular fibber-optic light is worrisome until the dye is washed out, considering the photosensitising qualities of ICG. Recent studies suggest possible photosensitising mechanisms, as there is a theoretical overlap between the emission of the light source and the absorption band of ICG (Gandorfer et al, 2003). There are data suggesting a shift of the absorption spectrum towards higher wavelengths when glucose 5% is used for ICG dilution instead of BSS, BSS plus, or viscoelastic. Other factors that might have an influence and should be evaluated include the actual concentration of ICG at the level of the ILM, as well as the role of an osmotic effect at the vitreoretinal interface after ICG molecules have bound to the ILM.

There is no doubt that better visualization of the ILM facilitates ILM peeling. There is no protocol for standardized intraocular ICG application concerning concentration, solvent medium, or exposure time. The impact of the duration of the illumination can only be evaluated in experimental settings, including morphologic or ultra structural analyses. The aim of our efforts should be the development of a standardized ICG solution with proven safety for intraocular application. Until then, the use of ICG to assist vitreomacular surgery should be limited to the more difficult cases, withy an emphasis on high dilution, short time application, and avoidance of illumination during application.

C-7.5. Types of Tamponade Used in Macular Hole Surgery

A retrospective case series reported by Thompson and associates (Thompson et al, 1996) found that the closure rate with 16% perfuoropropane (C_3F_8) was statistically significantly better than with lesser concentrations of C_3F_8. There were no significant differences in visual acuity among the three treatment groups.
A study without a control group of silicone oil tamponade without facedown positioning suggests that this technique may be an alternative for those patients who must travel or cannot maintain a facedown positioning. However, they must undergo a second operation to remove the silicone oil (Goldbaun et al, 1998).
In a comparative trial, Pertile and Claes compared silicone oil tamponade (n = 35 eyes) with SF_6 tamponade (n = 19 eyes) in patients with stage 3 or 4 holes. They found that 74% of patients in the silicone oil group had a postoperative best-corrected visual acuity of 20/50 or better compared with 47% of patients treated with gas tamponade (Pertile et al, 1999).

C-8. Conclusions

Macular hole surgery results in a flattening of macular hole edges in over 80% of patients. The evidence does not support surgery for patients with stage 1 holes. Some works supports surgery for stage 2 holes to prevent progression to later stages of the disease and further visual loss. For patients with stage 3 and stage 4 holes, surgery improves the vision in a majority of patients. Postoperative vision of 20/40 or better has been reported in 22% to 49% of patients in randomised trials. The risks of surgical complications include retinal detachment (3%), endophthalmitis (< 1%), cataract (>75%), and late reopening of the hole (2% to 10%). There is no strong evidence that adjuvant therapy used at the time of surgery results in improved surgical outcomes. Patient inconvenience, patient preference, and quality of life issues have not been studied.
Nevertheless, intentional ILM peeling, although a challenging procedure, can be considered safe. So far, there have been no reports of specific major complications causing permanent damage to the eye. In addition, ILM peeling seems to be an effective prophylactic measure against cellular reproliferation and recurrences of traction maculopathies such as epiretinal membranes and macular holes (Kuhn, 2002).

D - DIABETIC MACULAR EDEMA

Macular edema is a general response of the macula to any aggression and is seen in a wide variety of ocular and systemic diseases. Edema of the retina is defined as any increase of water of the retinal

tissue resulting in an increase in its volume – i.e., because of the structural organization of the retina, an increase in its thickness. Macular edema is, therefore, edema of the retinal tissue located in the macular area. This increase in water content of the macular tissue initially may be intra or extra-cellular. In the first case, also known as cytotoxic edema, there is an alteration of the cellular ionic exchanges with an excess of sodium ion inside the cell, In the second case, also called vasogenic edema, there is accumulation of fluid, predominantly extra-cellular, directly associated with an alteration of the blood-retinal barrier (BRB) (Cunha-Vaz et al, 1984). In this latter situation, Starling's law applies, and any loss of equilibrium among hydrostatic, oncotic, and osmotic pressure gradients across the BRB contributes to further movements and edema formation.
Diabetic macular edema is the leading cause of visual impairment in patients with diabetes mellitus. The Early Treatment Diabetic Retinopathy Study (ETDRS) defined clinically significant macular edema (CSME), if one or more of the following characteristics was present: (1) thickening of the retina at or within 500 μm of the centre of the macula; (2) hard exudates at or within 500 μm of the centre of macula, if associated with thickening of adjacent retina; and (3) a zone or zones of retinal thickening one disc area or larger, any part of which is within one disc diameter of the centre of macula (ETDRS, 1991).

D-1. Definition

Diabetic macular edema (DME) is retinal thickening caused by the accumulation of intraretinal fluid, primarily in the inner and outer plexiform layers, resulting from hyper permeability of the retinal vasculature. There are two patterns of macular edema: focal and diffuse. Focal macular edema is limited to well-defined areas of leakage, such as micro aneurysms. Diffuse macular edema is widespread and poorly demarcated leakage caused by more generalized disruption of the blood-retinal barrier. Macular edema involving one or both eyes has been shown to occur in approximately 29% of diabetic patients with duration of disease of 20 years or more (Klein et al, 1984). Of patients who develop macular edema, more than half will experience a loss of 2 or more lines of best-corrected visual acuity after 2 years of follow-up (Ferris et al, 1984).

D-2. Pathogenesis

The pathogenesis of diffuse DME is not fully understood. Mechanisms discussed include an imbalance between the filtration of fluid from the arteriolar end of the capillary bed and absorption of interstitial fluid at the venous end, an increase of retinal vascular permeability from micro aneurysms or by vascular permeability factors, such as vascular endothelial growth factor and interleukin-6 and traction of the posterior vitreous on the macula.
Clinically significant focal diabetic macular edema results from defined areas of retinal thickening caused by leakage from micro aneurysms. In the majority of these eyes, focal macular

photocoagulation effectively reduces clinically significant diabetic macular edema (CSDME) and preserves or improves best-corrected visual acuity. In a significant subset of patients, the CSDME is diffuse and often less responsive to macular laser photocoagulation (Shatz et al, 1976; Blankenship, 1979). Various factors have been shown to exacerbate diffuse DME, such as fluid retention caused by cardiovascular or renal disease, uncontrolled hypertension, pregnancy, and pan retinal photocoagulation (Bresnik, 1986).

In diabetic patients, DME is the most common cause of visual impairment. Although the precise pathogenic mechanisms of diffuse CSDME have not been established, abnormal permeability of the inner blood-retinal barrier is mainly due to the breakdown of this histological barrier. In addition, both clinical observations (Bresnick, 1994; Olk et al, 1993) and studies involving diabetic animal models (Krupin et al, 1982; Kirber et al, 1980) have implicated breakdown of the outer blood-retinal barrier in the development of diffuse DME. Although it is unclear how the posterior hyaloid contributes to the development of diffuse CSDME, condensation and contraction of the premacular hyaloid membrane cause tangential vitreomacular traction, which may increase the permeability of the retinal vasculature. Tangential traction may also induce or exacerbate an existing breakdown of the outer blood-retinal barrier. In addition to systemic factors, local factors may contribute to severe macular edema: excessive fluid leakage from micro aneurysms, capillary segments, or arterioles; tangential or vertical vitreous traction; and vascular leakage from early fibro vascular proliferation.

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The vitreomacular interface has been implicated in the pathogenesis of DME. Nasrallah and associates (1988) have shown that patients with DME have a lower incidence of posterior vitreous detachment (PVD) than do those without macular edema. In a prospective study (Hikichi et al, 1997) of 82 patients with late-onset diabetes and CSDME, Hikichi and associates reported spontaneous resolution of macular edema in 55 % of eyes with PVD and in only 25 % of eyes without PVD or with incomplete PVD. Therefore, traction may play a role in the formation and progression of diffuse DME, and PVD at the macula may support resolution of macular edema with subsequent restoration of visual acuity. Based on the earlier studies by Lewis and colleagues (1992) and Harbour and associates (1996), it was hypothesised that DME resulted from tangential traction by a thickened and still attached posterior hyaloid membrane, causing a very shallow macular detachment similar to the one observed in patients with macular hole (Lewis et al, 1992; Harbour et al, 1996). To date, it is not

clear whether traction is the primary cause of DME or whether vitreous changes caused by diabetic retinopathy have led to a secondary phenomenon of macular traction with exacerbation of macular edema (Lewis et al, 1992). One can imagine that the altered vitreous in diabetic eyes may stimulate epimacular fibrosis or proliferation of astrocytes (Messmer et al, 1998), which may use the ILM or posterior hyaloid as scaffold. This proliferation may ultimately lead to traction and aggravate capillary leakage into the macular area.

Structural changes of the vitreous gel, such as liquefaction and PVD, are associated with aging and the development of diabetic retinopathy (DR). Stitt and colleagues (1998) reported significantly increased levels of advanced glycation end products in the vitreous of patients with diabetes, forming increased irreversible cross-linking of vitreous collagen. Moreover, the ILM thickens with age, and an increase in the bilaminated pattern of fibronectin and laminin deposits has been observed (Kohno et al, 1987). Little is known about the molecular structure of the vitreoretinal interface in the eyes of patients with diabetes, but it seems unlikely that changes of the vitreous in these eyes would be limited exclusively to the vitreous gel. More likely, these changes may affect the vitreoretinal interface, including the ILM.

The premacular vitreous in diabetic eyes may also contain factors contributing to the persistence of macular edema. The presence of a posterior precortical vitreous pocket (PPVP) has been suggested to play a key role in determining the pattern of proliferation in some types of DR (Kishi et al, 1990; Kishi et al, 1993). Similarly, such a PPVP may also lead to a persistence of DME, through glucose-induced osmotic changes and effects on vitreous proteins. In addition, due to an altered blood-retinal barrier in DR, cytokines and growth factors (Kent et al, 2000) may have a local worsening effect. In support of this latter theory, it has been reported that the removal of vitreous with these inflammatory mediators in intraocular inflammation-related cystoid macular edema resulted in an improvement in visual acuity (Dugel et al, 1992). Vascular endothelial growth factor (VEGF) accelerates vascular permeability in addition to stimulating new vessel formation (Schlingemann et al, 1997; Tolentino et al, 1996), and patients with proliferative diabetic retinopathy have been shown to have an increase in the level of VEGF in the vitreous along with an increase in ocular permeability (Adamis et al, 1994; Aiello et al, 1994; Tanaka et al, 1997; Malecaze et al, 1994; Kent et al, 2000). Interleukin-6 also increases the permeability of the blood-retinal barrier (Kent et al, 2000; Bamforth et al, 1996).

A recent study with OCT showed three patterns of structural changes in DME: 88 % had a sponge-like retinal swelling, 47 % had cystoid macular edema and 15 % had a serous retinal detachment (Otani et al, 1999). Tangential traction by an (partially) attached posterior hyaloid membrane may play an important role. Yet, the ILM or an epiretinal membrane may also exert traction on the macula in diabetic patients, perhaps worsening edema in eyes that are already compromised due to vascular leakage and osmotic changes in the vitreous.

D-3. Evaluation of Macular Edema

D-3.1. Clinical Evaluation of Macular Edema

The clinical evaluation of macular edema is difficult. Direct ophthalmoscopy may show only an alteration of the foveal reflex. Stereoscopic fundus photography and slit-lamp biomicroscopy play an important role in demonstrating changes in retinal volume in the macular area, but successful use of these techniques depends on the observer's experience, and the results do not offer a true measurement of the volume change.

D-3.2. Measurements of Retinal Thickness in Macular Edema

New techniques that provide an objective measurement of retinal thickness recently have become available. Optical imaging instruments such as the Retinal Thickness Analyser (RTA) (Talia Technology, Ltd, Mevaseret, Zion, Israel) and Optical Coherence Tomography (OCT) Scanner (Zeiss Humphrey Systems, Pallo Alto, California), have been proposed as powerful tools for the objective assessment of macular edema. Both techniques are able to measure retinal thickness and rapidly generate thickness maps of the posterior pole, and both are non-invasive, non-contact procedures. Another instrument, the Heidelberg Retina Tomograph (HRT) (Heidelberg Engineering, Dossenheim, Germany), is a scanning laser ophthalmoscope that is able to measure retinal edema indirectly by performing a topographic assessment of an unevenly raised "retina", thus offering a map of relative increases in retinal thickness. The Retinal Thickness Analyser (RTA) (Cunha-Vaz et al, 2002) offers a quantitative and reproducible method to evaluate retinal thickness, particularly appropriate for measuring changes in retinal thickness in eyes with clear media and minimal macular changes. OCT, on the other hand, uses a unique cross-sectional scanning mode that offers highly accurate anatomic representation of the retina (Pedut-Kloizman et al, 1998), which is particularly useful when the retinal edema is associated with other pathologies.

D-3.3. Optical Coherence Tomography and Diabetic Macular Edema

The vitreomacular relationship and DME assessed by biomicroscopy, was shown to be insufficiently accurate to determine the status of the posterior hyaloid when it is only slightly detached from the macular surface, unlike Optical coherence tomography (OCT). Macular edema is the expression of many diseases and various treatments are being tested. Until recently, the methods available for assessing macular thickness were slit-lamp biomicroscopy and stereoscopic photography, which unfortunately do not give a quantitative measurement. OCT is indeed more sensitive than biomicroscopy in identifying vitreomacular adhesions (Gallemore et al, 2000) and allows earlier

diagnosis of shallow partial PVD (Gaudric et al, 1999; Haouchine et al, 2001). In addition, it allows precise assessment of macular thickness, with good reproducibility (Massin et al, 2001).

D-3.3.1. Retinal Thickness in Healthy and Diabetic Subjects Measured Using Optical Coherence Tomography Mapping Software

In recent years, new methods of measuring retinal thickness have been developed. Optical coherence tomography (OCT) is based on low-coherence interferometer and gives optical cross-sectional images of the eyes. It enables retinal thickness to be measured from tomograms by computer image processing. Since the commercialisation of OCT equipment (Humphrey Company, San Leandro, CA) several software's have been become available. A5 software can display a two-dimensional colour-coded map of retinal thickness in the posterior pole, and give the average retinal thickness of nine different areas of the macula. This software offers good reproducibility of retinal thickness measurements in normal subjects and in diabetic subjects with visual impairment due to macular edema. However, few data are available about the standard retinal thickness in different areas of the posterior pole in normal eyes, measured using this software.

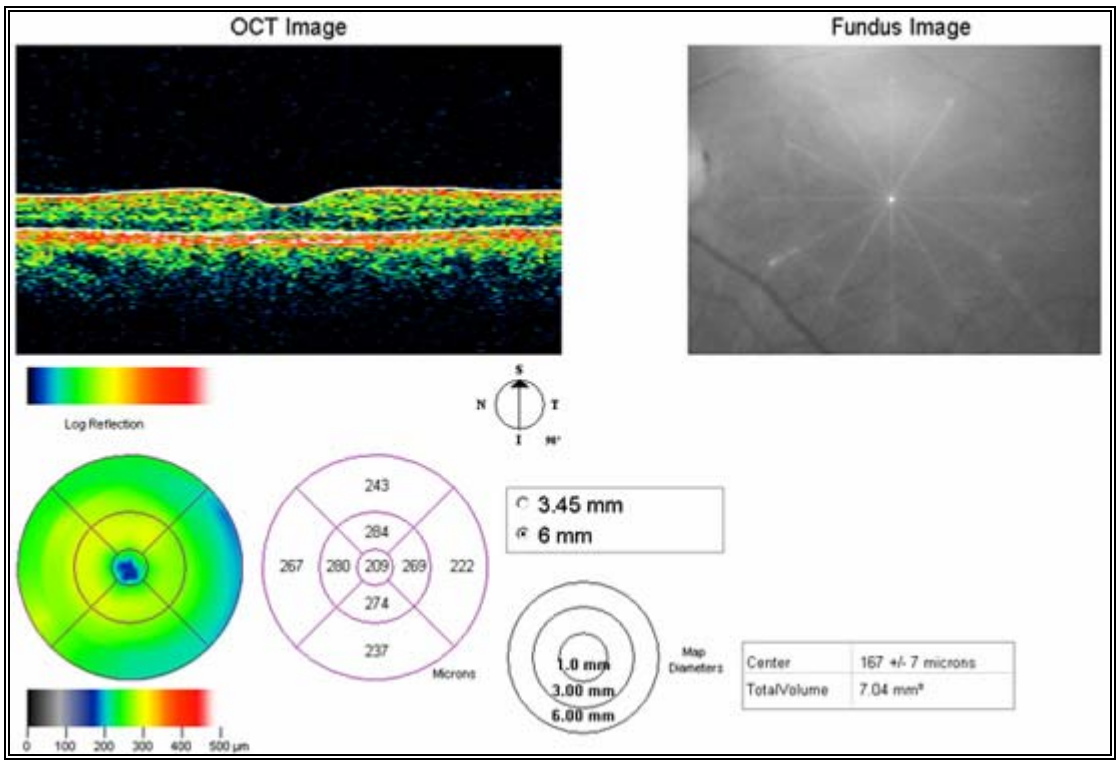


Figure 7 - OCT image of a normal foveal/macular area.

Massin and colleagues (2002) in a study with 60 healthy eyes and 70 eyes of 35 diabetic patients without macular edema on biomicroscopy concluded that OCT seems a sensitive tool for detecting

early retinal thickening. Retinal thickness was measured automatically with OCT mapping software. Mean retinal thickness was compared in subgroups of healthy patients based on age, sex, and eye, and in the eyes of diabetic patients and healthy subjects. In healthy subjects, mean retinal thickness in the central macular area 1000 μm in diameter was 170 ± 18 μm, which is very close to the 174 ± 18 μm reported by Hee and associates (Hee et al, 1998). There was no significant difference according to age, or left or right eye, but central macular thickness was significantly greater in men than women ($p = 0.0139$). No difference was observed between the eyes of healthy subjects and diabetic patients without macular edema on biomicroscopy, but OCT detected early macular thickening in 12 diabetic eyes.

D-3.3.2. Quantitative assessment of Retinal Thickness in Diabetic Patients with and without Clinically Significant Macular Edema Using Optical Coherence Tomography

Traditional methods of evaluating macular thickness, such as ophthalmoscopy or stereoscopic biomicroscopy, are insensitive to detecting small changes in retinal thickness. Current diagnostic instruments, such as laser-generated slit (Zeimer, 1998), and the Heidelberg Retina Tomographs (Ang et al, 2000), have been able to objectively assess macular edema but fail to show intraretinal structures. The advent of OCT offers the possibility of both high-resolution cross-sectional images of the retina and quantitative measurements of the retinal thickness (Hee et al, 1995; Huang et al, 1991). The technique has been applied in the diagnosis of a variety of macular diseases and in the evaluation of the treatment effects (Gaudric et al, 1999; Hee et al, 1995, 1998; Puliafito et al, 1995; Yang et al, 2001).

Yang and associates (2001), performed OCT in 14 eyes with DR and ophthalmoscopy evidence of clinically significant macular edema (CSME) and in 19 diabetic eyes without CSME. The mean ± standard deviation foveal thickness was 255.6 ± 138.9 μm in eyes with CSME, and 174.6 ± 38.2 μm in eyes without CSME ($p = 0.051$). The foveal thickness was correlated with visual acuity and the OCT identified sponge-like retinal swelling and/or cystoid macular edema in 11 (58%) eyes without CSME, and in 12 (86%) eyes with CSME. They concluded that criteria of CSME seem to be insufficient in really identifying macular edema. OCT may be more sensitive than a clinical examination in assessing diabetic macular edema and is a quantitative tool for documenting changes in macular thickening. Using OCT, Yang identified retinal swelling or cystoid macular edema in more than half (58%) of the eyes in the absence of ophthalmoscopy evidence of CSME. A recent study also showed that significant macular thickening could be detected by OCT in diabetic eyes even in the absence of CSME (Schaudig et al, 2000). Thus the ETDRS standard for defining CSME seems to be insufficient in really identifying macular edema. Alternatively, OCT may be more sensitive than a clinical fundus examination for early detection of intraretinal changes in diabetic macular edema.

Massin (Massin et al, 2003) in a retrospective study of 15 consecutive eyes that had vitrectomy for diffuse DME and OCT preoperatively and postoperatively, concluded that vitrectomy was beneficial in eyes with diffuse DME combined with vitreomacular traction but not in eyes without traction. OCT

allowed diagnosis of subtle vitreomacular traction and provided precise preoperative and postoperative assessment of macular traction. In seven eyes of six patients (group 1), vitrectomy was performed because of vitreomacular traction, observed on biomicroscopy or OCT. In the other eight eyes of seven patients (group 2), vitrectomy was performed for DME not responsive to laser photocoagulation, with no vitreomacular traction on biomicroscopy or OCT. The mean ± standard deviation (SD) follow-up after vitrectomy was 18 ± 10 months (range, 6 to 33 months): In group I, mean ± SD retinal thickness decreased significantly from 661 ± 181 µm preoperatively to 210 ± 32 µm at the end of follow-up (p = 0.018): Median best-corrected visual acuity (BCVA) improved from 20/100 before surgery (range, 20/250 to 20/50) to 20/80 at the end of follow-up (range, 20/250 to 20/25; p = 0.046). In one eye in-group 1, vitreomacular traction was only observed on OCT and not on biomicroscopy. In-group 2, mean ± SD retinal thickness decreased from 552 ± 103 µm preoperatively to 428 ± 121 µm at the end of follow-up (p = 0.2). Median BCVA was 20/100 before vitrectomy (range, 20/320 to 20/63) and 20/200 at the end of follow-up (range, 20/250 to 20/63; p = 0.78). The OCT findings and the postoperative evolution observed in group 1 confirmed the hypothesis of Lewis and colleagues (Lewis et al, 1992), who suggested that the taut thickened posterior hyaloid exerted tangential vitreomacular traction that induced or exacerbated DME. The thickening of posterior hyaloid may have been partly due to the structural changes in the vitreous cortex reported in diabetic patients (Sebag et al, 1992; Stitt et al, 1998) and to infiltration of the hyaloid by cells of glial and epithelial origin (Jumper et al, 2000). In the eight eyes of group 2, macular edema was not combined with vitreomacular traction on OCT. The posterior hyaloid did not thicken; it was either not visible on OCT or it was slightly detached from the posterior pole, indicating an early stage of partial PVD, as described by Uchino (Uchino et al, 2001). In those eight eyes, the final results at 17 months did not constitute a significant benefit as regards either BCVA or the decrease in retinal thickness. Three months after surgery, they observed a decreased in retinal thickness, from 522 ± 103 µm to 363 ± 123 µm, which was of borderline significance, but in most eyes it was transient. This transient decrease may be due to vitrectomy and blood pressure control, because no significant change in retinal thickness was observed in the no operated on fellow eyes. Several explanations have been suggested for postvitrectomy improvement of diabetic macular edema in the absence of vitreomacular traction. The vitreous may act as a potential reservoir of inflammatory substances or growth factors such as VEGF, which promotes vascular permeability (Aiello, 1997), and its removal by vitrectomy may improve diabetes macular edema. Another explanation is that vitrectomy may improve oxygenation of the retina (Stefansson et al, 1990). OCT proved to be a useful tool that provided precise objective assessment of macular thickness before and after vitrectomy for diffuse DME. Although this rare condition can be suspected on biomicroscopy in most cases, OCT confirms the diagnosis by providing an objective image of vitreomacular traction; in addition, OCT may help to detect more subtle traction not visible on biomicroscopy.

In conclusion, on OCT examination, macular edema appears as a collection of hypo reflective spaces within the retina and serial OCT examination allows more accurate qualitative follow-up of the changes in retinal profile than biomicroscopy alone, and extending this to retinal thickness measurement or mapping, an objective quantitative assessment of small changes during follow-up is possible (Thomas

et al., 2004). FA is extremely useful in demonstrating macular edema, but it is qualitative and dependent on the source of leakage persisting in order for the dye to leak into the area of edema to highlight it. OCT on the other hand shows the edema that is present whether the focus is still leaking or not and is also quantitative. The radial line option on the OCT software takes six radially disposed scans centred on fixation, and thus the retinal thickness can be calculated for 600 macular points. The software allows for a false-colour retinal map to be reconstructed from the data, enabling the average thickness to be displayed in each of nine macular zones, and also a calculation of the macular volume. Thus a quantitative assessment can be made allowing longitudinal follow-up assessment of spontaneous resolution or response to any treatment. OCT can also have an advantage over FA in being able to image the Z-plane in addition to the X-Y-plane (Thomas et al., 2004). Z-plane imaging is useful in describing the morphological appearance of diffuse DME. A recent OCT study has shown two patterns of DME, that is, a domed appearance where the fluid everts the fovea and a diffuse appearance where the inner retinal contour is flat (Massin et al., 2003). Another study describes three patterns of intraretinal edema in diabetic patients: sponge-like retinal swelling, which most commonly, cystoid macular edema and serous detachment, which may coexist with either of the first two patterns (Otani et al., 1999).

D-4. Surgical Treatment

The treatment of DME is mainly based on laser photocoagulation. The Early Treatment Diabetic Retinopathy Study Research Group (ETDRS, 1985) showed that, in eyes with DME, focal laser photocoagulation reduces the risk of moderate visual loss by 50% or more, decreases the frequency of persistent macular edema, and increases the chance for improvement in best-corrected visual acuity. However, 15% of eyes experienced moderate visual loss after 3 years of follow-up despite focal laser photocoagulation (ETDRS, 1985). Diffuse DME is a more complex therapeutic problem than is focal macular edema. Grid-pattern laser photocoagulation has been shown to result in resolution of macular edema in 68 % to 94 % of patients (Olk et al, 1986; Lee et al, 1991). In a more recent study involving 302 eyes with diffuse DME, modified grid laser photocoagulation stabilized or improved best-corrected visual acuity in 75.4% of eyes, while 24.6% of eyes experienced loss of vision after 3 years of follow-up (Lee et al, 1991).

Although vitrectomy also occasionally causes severe complications, the complications from simple vitrectomy has been reduced by recent improvements in surgical technique and instrumentation. Because vitrectomy, unlike photocoagulation, does not cause irreversible retinochoroidal impairment, the procedure, if it is effective, should be included among the alternative treatments for patients with DME.

The results of vitrectomy in DME have been positive since Lewis, were the first to describe their findings in 1992 (Lewis et al, 1992). Others studies have reported favourable anatomic and functional results after vitrectomy and removal of the posterior hyaloid and tractional forces associated with a thickened and taut premacular hyaloid in patients with macular edema (Lewis et al, 1992;

VanEfferente et al, 1993; Harbour et al, 1996; Tachi et al, 1996; Pendergast et al, 1998). The surgical procedure consisted of removal of the posterior hyaloid, including peeling of epiretinal fibro cellular proliferations; the inner limiting membrane (ILM) was not approached. Although is unproven, tangential traction may play a role in the formation and progression of diffuse DME associated with advanced vitreoretinal interface disease (Gandorfer et al, 2000).

Gandorfer and colleagues (2000) evaluate the surgical results of pars plana vitrectomy with peeling of the inner limiting membrane (ILM) from the macula in a series of 12 eyes with diffuse DME. Intraoperative, the posterior hyaloid was found thickened and completely attached to the macula in 10 eyes. Postoperatively, retinal thickening resolved or decreased in all eyes and visual acuity improved by at least two lines in 11 eyes. Best-corrected visual acuity developed within 4 to 12 weeks.

Yang (Yang, 2000) analysed the surgical results of eyes with massive hard exudates secondary to DME treated with combined pars plana vitrectomy, posterior hyaloid removal, focal endolaser treatment, and pan retinal photocoagulation. The author retrospectively analysed the surgical outcome of 13 consecutive eyes with massive diabetic macular exudates that had had at least one session of focal and/or grid laser treatment without any effect. All 13 eyes showed significant decreases in macular edema and hard exudates, a process that became clinically obvious 3 months after the operation. Eleven eyes had improved vision of at least two lines during an average follow-up period of 14.8 months.

D-5. Conclusions

DME is one of the main causes of visual impairment in patients with DR. Because the incidence of macular edema is higher in eyes without a spontaneous PVD than in those with one (Hikichi et al, 1997; Nasrallah et al, 1988; Schepens et al, 1984), and because the traction of the vitreous cortex on the macula plays an important role in the exacerbation of macular edema, vitrectomy has been performed to separate the posterior hyaloid from the retina to treat macular edema. This release of macular traction helps resolve the edema (Lewis et al, 1992, Harbour et al, 1996; Tachi et al, 1996; Pendergast et al, 2000; Gandorfer et al, 2000: Lewis, 2001; Yamamoto et al, 2001).

In many macular conditions, OCT may be diagnostic (for example in MH) and used as a non-invasive alternative to FA. However, even in conditions that are readily diagnosed biomicroscopically, such as DME, the objective serial quantitative measurement offered by OCT is of value in follow-up since treatment is variably effective and difficult to assess by biomicroscopy or FA.

III. OBJECTIVES

The main objective was ***to study the role of the internal limiting membrane (ILM) extraction during pars plana vitrectomy (PPV), in the surgical treatment of eyes with macular hole (MH) and chronic diabetic macular edema (CDME).*** To achieved this objective, we analysed:

1. Prospective clinical follow-up of one year, of 28 patients with MH and CDME, submitted to PPV with ILM extraction.
2. Clinical success rates of the extraction of the ILM during PPV for surgical treatment of MH and CDME.
3. The technical procedure of the ILM extraction.
4. Contribution of the extraction of the ILM, in the clinical resolution of the macular edema in the diabetes maculopathy after PPV.
5. The value of the OCT, in the follow-up of patients with macular edema due to diabetic retinopathy and MH.

The secondary objective was ***to establish a clinic-pathological correlation by the histological study of the ILM, dissected in eyes with MH and diabetic macular edema (DME), during PPV***. This included:

1. Histological findings and differences of the ILM observed by transmission electron microscopy (TEM) and light microscopy (LM), in two types of macular diseases: isquemic (DME) and non-isquemic (MH) retinal disorders.
2. The contribution of ILM histological analysis for better understanding the physio-pathogenesis of MH.

IV. MATERIAL AND METHODS

A. Patients and Methodology

A-1. Study Design

This was a prospective one-year follow-up study, non-randomised, non-comparative, with two groups of patients, with two different types of maculopathy. The clinical approach was done at the Instituto de Microcirugía Ocular de Barcelona.

Approval by the local ethics committee was obtained, before starting the recruitment. The study was conducted according to the principles contained in the Declaration of Helsinki (http://www.arvo.org/AboutARVO/helsink.asp.) and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (http://www.ncehr-cnerh.org/english/gcp). All the subjects enrolled on the study gave informed consent. The compliance of the study was 100 %, with all the patients observed until the end of the follow-up.

A-2. Study Population and Patient Eligibility

The patients were submitted to an ophthalmologic examination before surgery and during one year in the post-operative time, after PPV with extraction of the ILM. All patients were divided in two groups: 1) group of macular holes (MH); 2) group of diabetic macular edema (DME) with CDME resistant to laser treatment.

In the MH group, the inclusion criteria were, macular holes stages 2 – 4, as Gass classification (Gass et al, 1988). For this group, the exclusion criteria were: MH stages I, glaucoma, diabetic retinopathy, age related macular degeneration and retinal detachment.

In the DME group, the inclusion criteria were patients with CDME unresponsive to a laser treatment. The patients had CSDME, as ETDRS classification (ETDRS, 1991), chronic and resistant to previous photocoagulation or other treatment. In all of the eyes, there was no ophthalmoscopy or OCT examination evidence of traction from a posterior hyaloid membrane or from proliferative tissue. The exclusion criteria were diabetes retinopathy without CSDME, iris neo-vascularization, haemovitreous, glaucoma, age related macular degeneration, diabetic macular holes, cystoids macular edema following diabetic cataract surgery, central serous coroidopathy and macular/retinal detachment.

A-3. Evaluation Performed and Outcome Measures

The patients were submitted, before surgery and after PPV, to an ophthalmologic examination consisted in: objective refraction, best-corrected Snellen visual acuity, intraocular pressure measurement, biomicroscopy, fundus examination and indirect ophthalmoscopy in mydriasis.

Postoperative follow-up was performed during the first year at one day, one week, 1, 3, 6 and 12 months after surgery. In the preoperative and postoperative periods, digital fluorescein angiography for DME group, posterior pole fundus photography (30º central: macula, optic nerve and vascular arcades) and macular mapping with optical coherent tomography (OCT; Humphrey model 2000; Humphrey Instruments, San Leandro, CA) were performed as complementary diagnostic examinations. The schedule used for the study was represented in Table III.

Table III – Schedule of study visits. The procedures used for the study were: visual acuity (VA), intraocular pressure (IOP), biomicroscopy (Biom.), fundoscopy (Fund.), indirect ophthalmoscopy (IO), digital fluorescein angiography (FA; only in DME group); fundus photography (FP) and optical coherence tomography (OCT).

	Before Surgery	After Surgery					
		Day 1	Day 7	1 Month	3 Month	6 month	12 month
VA	√	√	√	√	√	√	√
IOP	√	√	√	√	√	√	√
Biom.	√	√	√	√	√	√	√
Fund.	√	√	√	√	√	√	√
IO	√	√	√	√	√	√	√
FA	√					√	√
FP	√			√	√	√	√
OCT	√			√	√	√	√

The OCT was essential to measure the foveal thickness before surgery and during the follow-up period in the DME group, and for the diagnosis, classification (Figure 8), and closure analysis progression of macular holes in MH group. Figure 8 showed one enrolled eye with MH, before surgery.

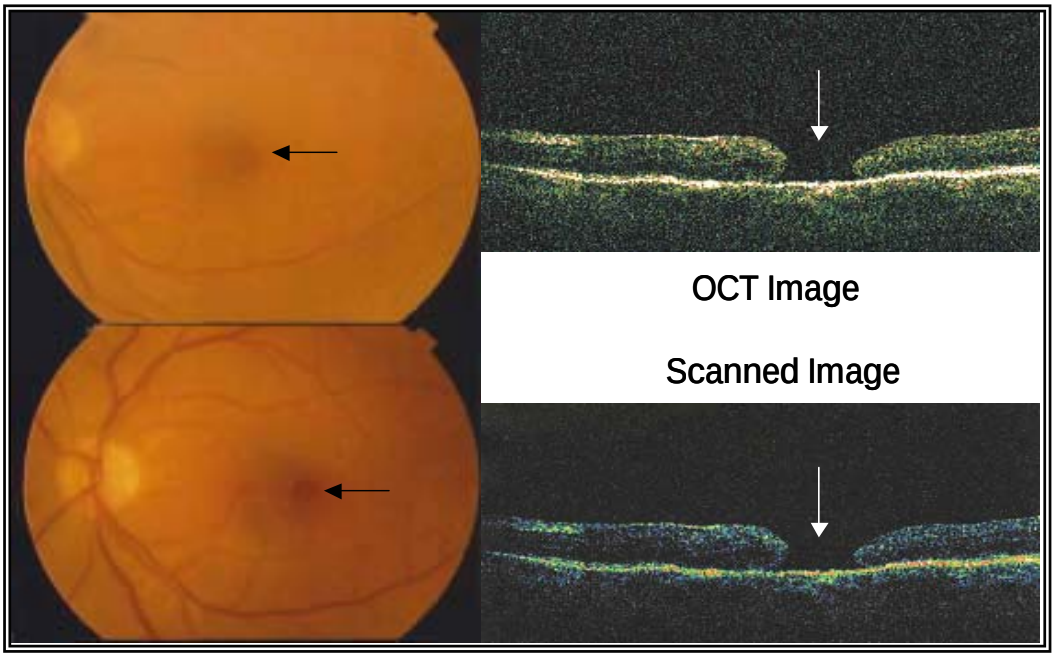


Figure 8 – One enrolled left eye in MH group. MH stage 4 observed with FP, OCT image and OCT scanned image analysis, in preoperative period.

In DME group, macular edema was diagnosed by fundus examination, fluorescein angiography, and OCT. Digital fluorescein angiography (FA) with IMAGE net system (Topcon, Tokyo, Japan) was performed at baseline and postoperative time (6 and 12 months follow-up). FA was performed with an intravenous injection of 5 ml of 10% sodium fluorescein in water, and images were captured for up to 10 minutes after dye injection. The extent and types of dye leakage and the amount of fluorescence was evaluated with reference to the baseline angiograms. Figure 9 showed one case of DME in preoperative time.

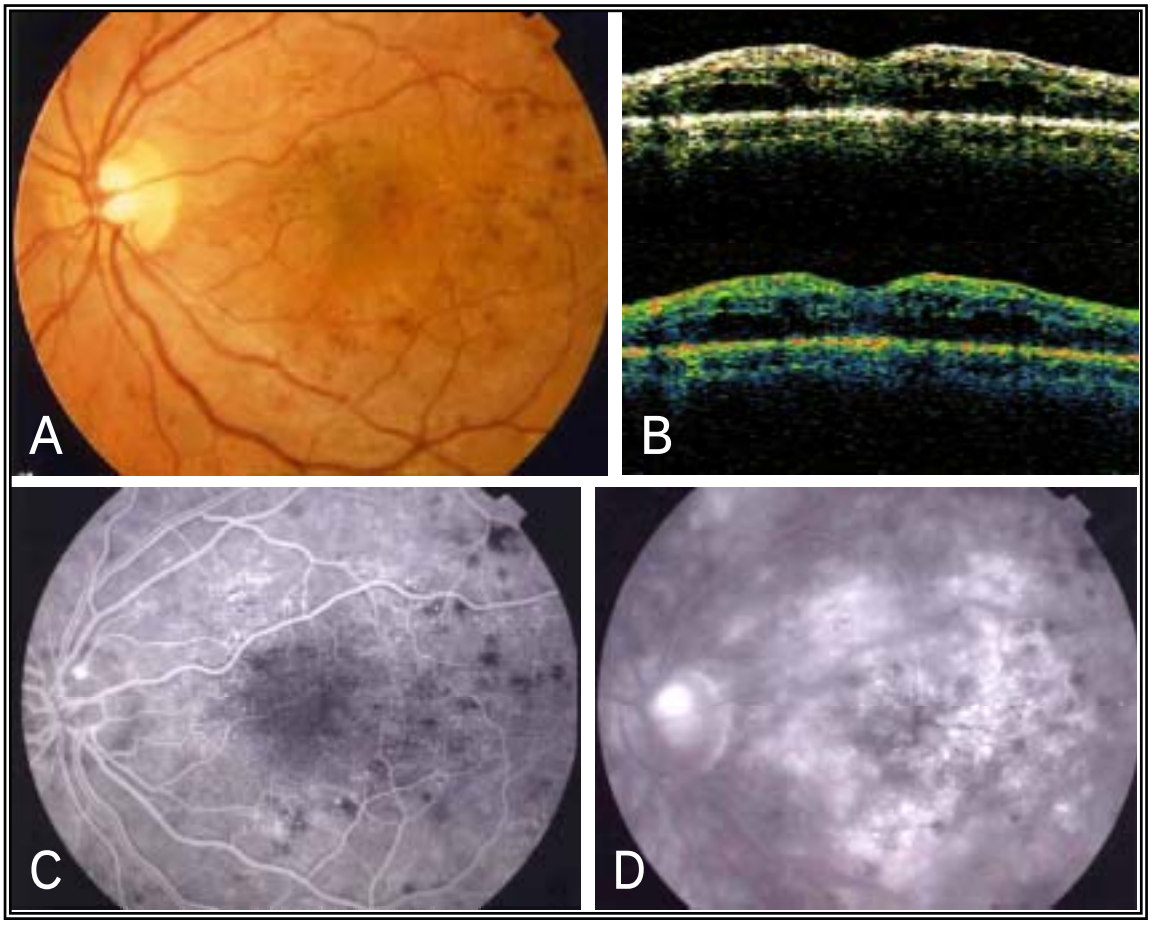


Figure 9 – One enrolled left eye in DME group. In FP we observed CSDME (A) and OCT (B) showed thickened macular edema with evident sub-retinal fluid. FA showed micro aneurysms, haemorrhages and hard exudates in the posterior pole at initial stage (C). At final stage remarkable diffuse CSDME was showed (D).

The main outcome measure in the DME group was the change in central macular thickness (CMT). CMT was defined by the average thickness of a central macular region 1000 μm in diameter, centred on the patient's foveola, and automatically measured by OCT. OCT mapping was performed using commercially available equipment and done through a dilated pupil by an experienced examiner who was aware of the clinical findings for each patient. The OCT examination comprised 6 radial 6-mm-long scans of each eye, centred on the patient's fixation point, at 30º, 60º, 90º, 120º, and 150º. Retinal

thickness was computed automatically, using retinal mapping software. This mapping averaged the 6 scans to give the CMT in a central area 1000 μm in diameter (i.e. the average of 100 measurements). The closure or recurrence of the macular holes was diagnosed by contact or non-contact (78 D) lens slit-lamp biomicroscopy and OCT macular analysis.

B. Surgical Procedure

All patients were submitted to pars plana vitrectomy (PPV), and during the surgical treatment the main objective was the epiretinal dissection and the extraction/peeling of the ILM.

The patients were operated under regional anaesthesia (retrobulbar block), after pharmacological mydriasis. Before the extra-ocular muscles block, almost all the patients were submitted to some medication with ondansetron (4 mg), midazolam (1-4 mg) and atropine (0,5 mg) via venous catheter, under anaesthesiologist supervision and management. With the help of intravenous propofol bolos (2-4 mg/Kg) and after skin and lids iodo-povidona solution meticulous disinfections, 5 ml. of local anaesthetics mixture (lidocaíne 2%, mepivacaíne 2% and/or rubivacaíne 0,75% solutions) was injected into the retrobulbar space. According to anaesthesiologist experience and indication, the analgesia was potentate during the surgery, with the help of venous fentanest (0,05-0,10 mg) and/or perfusion of remifentanil (0,01-0,1 μg/Kg/min.). During the surgical procedure, all the patients had cardiac (pulse, arterial pressure, and electrocardiogram), pulmonary and oxygen saturation monitorisation, under anaesthesiologist control.

Surgery was performed with monitored anaesthesia care. All the cases were submitted to a standard PPV using three sclerotomies, began at the centre and go until the posterior pole and finally to the periphery. If the cortical vitreous was observed adhering to the posterior pole, the posterior hyaloid was dissected from the retina by using the vitreous probe (Accurus 800 XS4, Alcon, Fort Worth, Texas, USA) or a silicone-tipped cannula under active aspiration. Posterior vitreous separation was confirmed by elevation of the peripapillary glial ring. Dissection began over the optic disc or the temporal vascular arcade, followed by removal of the hyaloid and posterior cortical vitreous peeled across the macula and into the periphery 360º. A complete vitrectomy was performed as far peripherally as safely possible. Twenty-five milligrams of indocyanine green (ICG) was dissolved in 10 ml of distilled water, and 0,3 ml of this solution was mixed in 1 ml of sodium hyaluronate viscoelastic with a low molecular weight (600,000-1.200,000). The final concentration of the ICG solution was approximately 0.06%. Careful was take to purposefully avoided injecting the ICG directly into the macular hole and redirected the internal light pipe while the ICG was in the eye. The posterior ILM was nicely stained with 0.2 to 0.3 ml of the ICG solution and after few seconds, according to surgeon experience, washout with BSS solution and vitreous probe aspiration. A sub-ILM incision was made using a sharp pick, begun approximately 500 to 1000 μm from the fovea and dissected circumferentially in a continuous circular manner at the macular area corresponding to 2-3 optic disc diameter (the foveal centre was included in this area) by using asymmetric Tano forceps (Synergetics Inc., St. Charles, MN). To eliminate the ICG in the vitreous cavity and to decrease the risk of retinal

toxicity induced by the ICG, the vitreous probe or a silicone-tipped cannula was used to aspirate the residual ICG. The retinal periphery was inspected for retinal breaks, and any breaks found were treated with either cryopexy or laser. A retinal scattered pan-photocoagulation in pre-proliferative or proliferative status and criotherapy transcleral at the 180º superior periphery was performed in all diabetic patients. In the macular hole group, a fluid-air exchange was performed and 20 to 25% of hexafluoret (SF_6) was injected at the end of the procedure. In the MH group, the patients were instructed to maintain a facedown position more than 90% of the time, for a minimum of 5 days.

C. Histopathological Analysis

During the surgical treatment, epiretinal dissection and the extraction/peeling of the ILM, were done with appropriate intra-ocular vitreous forceps. The ILMs of MH and DME groups were prepared for light microscopy (LM) and transmission electron microscopy (TEM) analysis. All the histological data were recorded and the differences were compared.

Specimens were immediately fixed in 2,5% glutaraldehyde and 2 % paraformaldehyde in 0.1 M phosphate buffered saline (PBS), as described previously (Casaroli-Marano et al, 1995). For conventional electron microscopy, specimens were rinsed in PBS 0.1 M pH 7.4, post-fixed in 1 % osmium tetroxide phosphate buffered solution for 1 hr, dehydrated in a graded acetone series, and then embedded in resin for polymerisation at 60º C. Semi thin (0,5-2 µm) and ultra-thin sections (50-75 nm) were obtained by conventional ultramicrotomy (OmU2, Reichert-Jung, Austria). Ultra-thin sections were placed on copper grids (200 mesh), and contrasted with uranyl acetate and lead citrate solution for TEM (Jeol 1010 EMT, Japan). Semi-thin sections were contrasted with tolouidine blue and observed in LM adapted with digital system for capture images.

The histological control group consisted by retinas obtained from 2 donated eyes without any ophthalmic disease. Those normal human eyes, donated for corneal transplant in accordance with the Standardized Rules for Development and Applications of Organ Transplant, as defined in Portuguese law, were obtained from the Eye Bank of the Ophthalmology Department of the Coimbra University Hospital, in Coimbra, Portugal.

In TEM images of the normal retinas observed from macula, equator and periphery, the sections photographed were projected and 75 measurements of ILM thickness (25 measurements, for each retinal region: 5 measurements in 5 photographs) were performed by random superposition of an unbiased test-volume frame (4,23 mm^2/test point). The same 75 measurements were done in various ILM, of diabetic and MH specimens, in macular area, using TEM images.

D. Statistical Analysis

Two-paired *t*-student test was used to estimate statistical differences between the variability of frequency for the same group of patients. A comparison of scores in different visits was done, and

variables were analysed. A value of $p < 0.05$ was considered significant. Clinical comparisons between the two groups were not done. By other hand, histological differences were analysed between the groups and the control eyes, especially when analysed the ILM thickness and the cellular types and others histopathological aspects.

V. RESULTS

A. Macular Hole Group

A-1. Patients

In the MH group with ten patients, there were two men (20 %) and eight women (80 %). The mean age was 60 ± 19 years old, with the younger patient with 14 years old (traumatic hole) and the older with 73 years old (idiopathic hole). Assessing the fovea with OCT, six eyes (60 %) had type 2 and 3 macular holes and four eyes (40 %) had type 4, according to Glass classification. In this group, five patients presented idiopathic macular holes (50 %), three were macular hole with epiretinal membrane (30 %), one was myopic macular hole (10 %) and one was traumatic macular hole (10 %).
Clinical characteristics, such as age, sex, type of MH, and VA achieved during the follow-up, were reported in Table IV.

Table IV. Clinical characteristics of the patients enrolled in MH group.

Patients	Age (years old)	Sex (M / F)	MH Classification	Visual Acuity (months) 0	1	3	6	12	Type of MH
1	60	Female	3	0,16	0,5	0,5	0,4	0,5	Idiopathic
2	67	Female	2	0,3	0,3	0,4	0,2	0,6	ERM
3	70	Female	3	0,1	0,06	0,4	0,4	0,4	ERM
4	38	Female	4	0,08	0,08	0,08	0,08	0,08	Myopic
5	71	Male	2	0,2	0,125	0,3	0,3	0,3	Idiopathic
6	73	Female	3	0,2	0,4	0,4	0,5	0,5	ERM
7	71	Male	2	0,25	0,16	0,2	0,3	0,3	Idiopathic
8	66	Female	4	0,06	0,05	0,06	0.08	0,05	Idiopathic
9	70	Female	4	0,05	0,2	0,1	0,1	0,2	Idiopathic
10	14	Female	4	0,05	0,16	0,2	0,2	0,2	Traumatic

ERM: epiretinal membrane; M: male; F: female; shaded columns, corresponds to patients who didn't improved initial VA.

A-2. Postoperative Results

All eyes were submitted to pars plana vitrectomy (PPV) with ILM extraction, for surgical treatment of MH stages 2 – 4. Fifty percent of the eyes (5 eyes) had nuclear lens opacification, at the beginning of the study. During follow-up, 4 eyes (40 %) were submitted to phacoemulsification with implantation of an intraocular lens in the capsular bag, because of the evolution in the lens nuclear sclerosis. Three eyes (30 %) were operated between the first and second months of follow-up and another one (10 %) at third month. The mean ± standard deviation (SD) of VA was 0.14 ± 0.08 before surgery, 0.20 ± 0.15 at one month, 0.26 ± 0.15 at three month, 0.27 ± 0.14 at six month and 0.31 ± 0.18 at twelve month of

the follow-up. VA achieved during follow-up, was showed in Figure 10. All patients, except one (patient 8), maintained or improved the best-corrected VA that was situated between 0.05 and 0.6, at the end of the study.

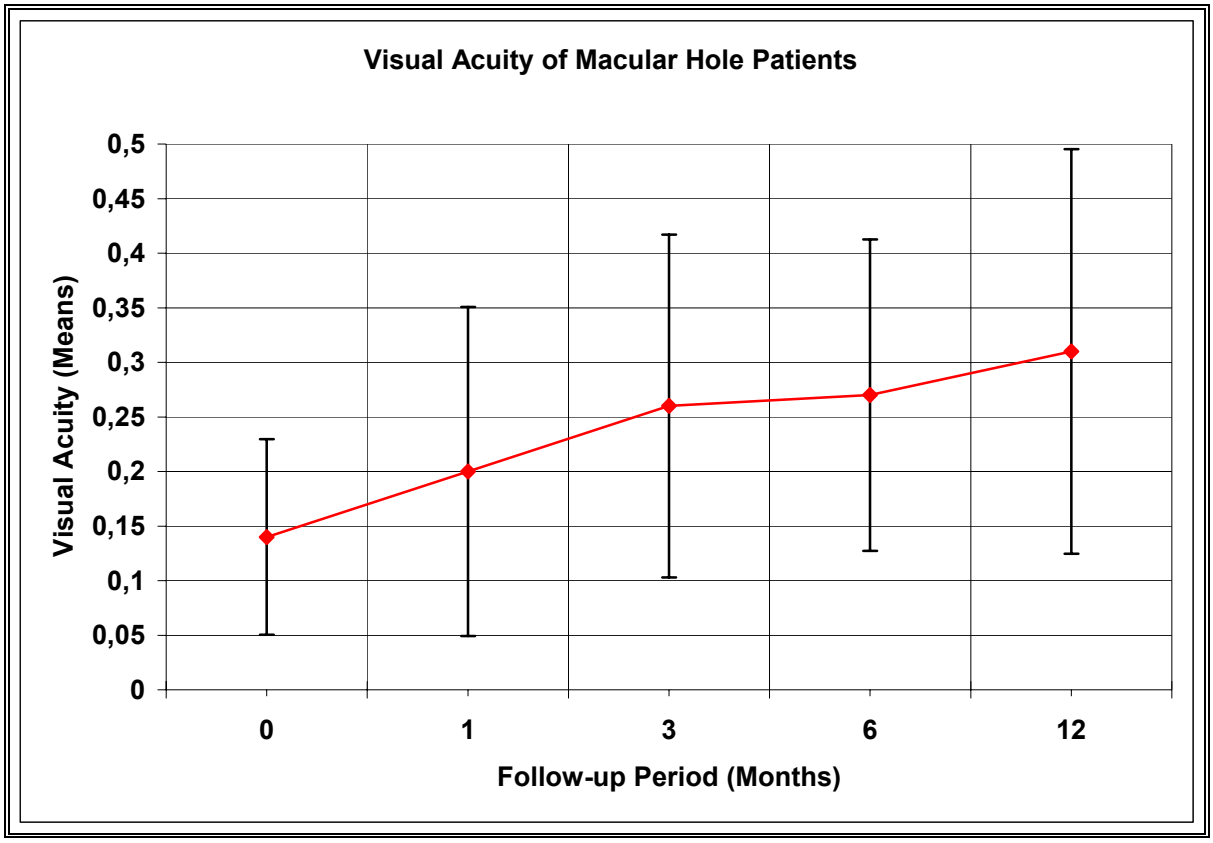


Figure 10. VA obtained in the MH group. Improvement of VA was observed in 8 patients after surgery. The mean ± SD of VA was showed.

To analyse and compare the progression of VA in MH group, we applied the two-tailed *t*-student to study the samples. Statistical analysis showed that the mean of postoperative VA at the twelve month of follow-up was significantly better than those registered in preoperative time ($p = 0.003$). Moreover the mean of postoperative VA at the twelve-month follow-up was significantly better than those achieved at one month after surgery ($p = 0.024$). The improvement of the VA was statistically significant only after the third month from surgery. The Figure 11, showed the significant recovery of VA, during follow-up.

No complications were attributed to the use of ICG, which appears to be a safe adjunct to macular hole surgery.

In the two patients who didn't had a better VA at the end of follow-up (patients 4 and 8), the histological analysis by TEM, showed sensory retinal components on the retinal side of the ILM.

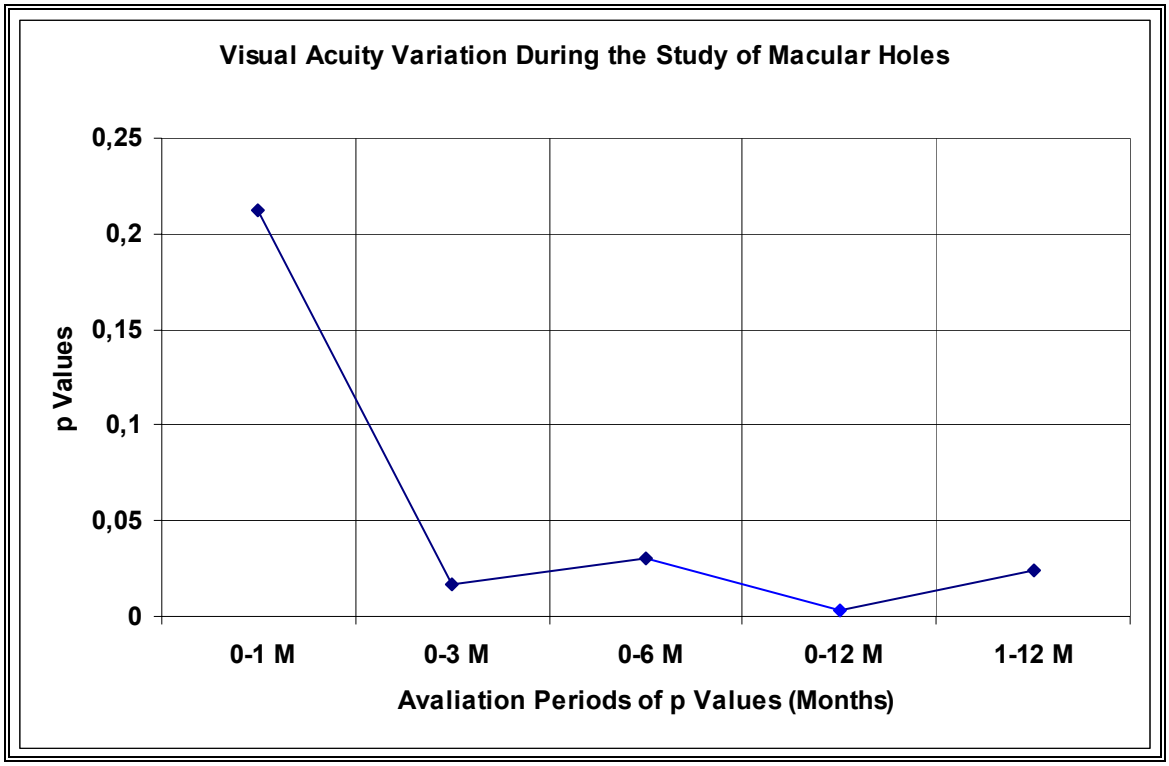


Figure 11. VA recovery in the MH group. Statistical signification of VA variability was evidenced after the third month of follow-up.

A-3. OCT Results

To follow the anatomical results after surgery, we examined the patients by using OCT in the 1st, 3rd, 6th and 12th months of follow-up. The OCT follow-up of those eyes showed that the macular hole closure was achieved in nine (90 %) of them, with one reopening macular hole in 10th month after surgery (patient 8). The patient 8 did not improve her final VA.

In all eyes enrolled in this group, the OCT was able to accompanying the anatomical evolution of the hole closure (Figure 12).

Quantitative information was been extracted directly from the OCT preoperative images, including the type and the grossly diameter of the hole, and used as a diagnosis approach.

The evaluation and evolution of the surrounding sub-retinal fluid accumulation (Figure 12), was always achieved during the study.

The high resolution obtained in the tomographs was very useful to monitoring hole progression and anatomic evolution after surgery, in all patients.

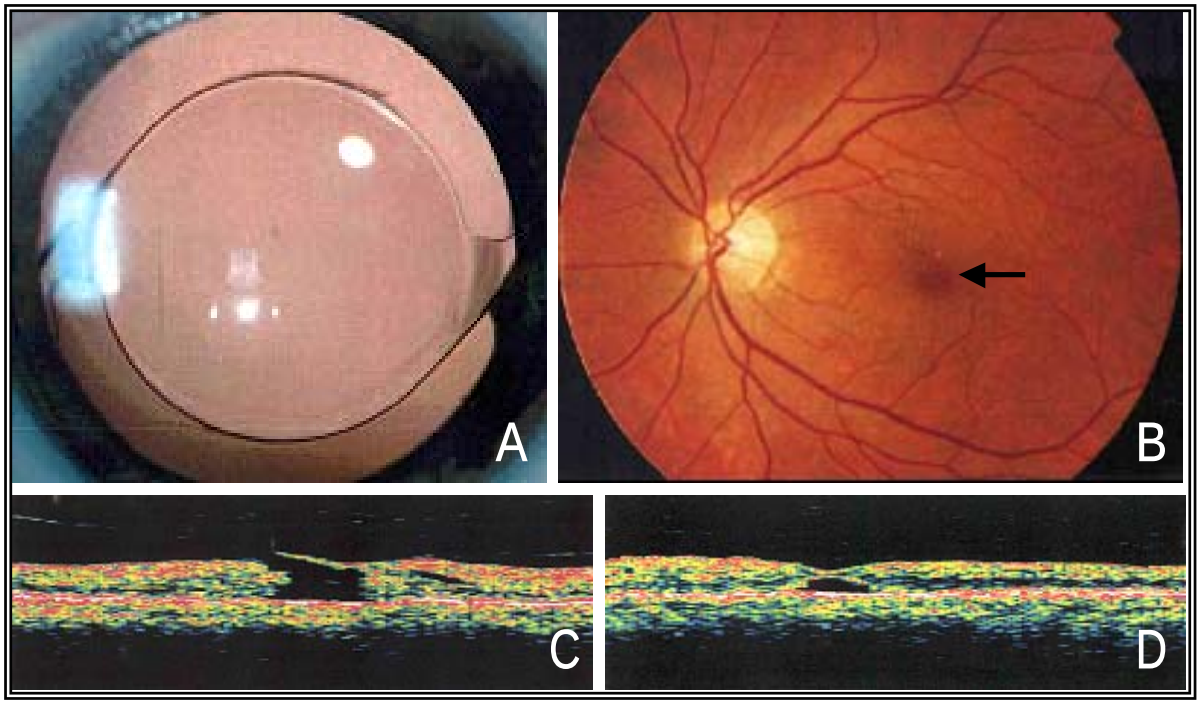


Figure 12 – Anatomical evolution of MH after surgery. In a pseudo-phaquic eye (A), we observed a MH (arrow) by using FP (B). Preoperatively OCT of the same eye, confirming the diagnosis and was used to classify the MH, in stage 2 (C). Postoperatively (1 month), the OCT (D) was very useful to study the anatomic evolution of the MH.

B. Diabetic Macular Edema Group

B-1. Patients

In 18 DME patients, thirteen were men (72 %) and five were women (28 %), with a mean age of 57.54 ± 14.6 years old, between 25 and 82 years old. All patients except two, had type II diabetes and fifty percent had arterial hypertension. The mean diabetes duration was 17.05 ± 6.63 years. Fifteen eyes (83 %) had undergone retinal pan-photocoagulation and macular photocoagulation, for proliferative diabetic retinopathy with CDME. Three (17 %) were submitted to macular photocoagulation to treat diabetic macular edema, before surgery. At the beginning of the study, there were 10 eyes (56 %) with biomicroscopic findings of cortical cataracts at initial stage, one eye was aphaquic, and one eye was previously submitted to cataract surgery, with intraocular lens on the capsular bag. The clinical characteristics of DME patients enrolled in the study were represented in Table V.

Table V. Characteristics of patients in DME group.

Patients	Age (years old)	Sex (M / F)	Foveal Thickness		Visual Acuity			(months)	
			0	12	0	1	3	6	12
1	25	Male	800	261	0,2	0,2	0,125	0,3	0,5
2	25	Male	660	479	0,3	0,4	0,4	0,4	0,4
3	62	Male	524	148	0,06	0,06	0,125	0,06	0,1
4	66	Male	380	263	0,2	0,1	0,4	0,4	0,4
5	36	Female	400	286	0,2	0,3	0,4	0,4	0,5
6	63	Male	459	298	0,125	0,08	0,16	0,3	0,2
7	49	Male	751	232	0,06	0,1	0,1	0,6	1
8	65	Male	534	298	0,2	0,3	0,325	0,3	0,4
9	67	Female	574	237	0,16	0.06	0,2	0,2	0,2
10	62	Female	345	205	0,2	0,16	0,01	0,02	0,2
11	82	Male	597	569	0,1	0,06	0,06	0,06	0,08
12	64	Female	418	196	0,2	0,25	0,25	0,25	0,32
13	64	Female	525	197	0,3	0,32	0,4	0,3	0,32
14	64	Male	575	161	0,02	0,06	0,03	0,03	0,03
15	64	Male	571	350	0,2	0,1	0,25	0,16	0,16
16	58	Male	412	367	0,06	0,05	0,06	0,08	0,06
17	58	Male	417	424	0,1	0,06	0,06	0,08	0,06
18	69	Male	657	299	0,06	0,06	0,16	0,1	0,25

M: male; F: female; shaded columns, corresponds to patients who worst initial VA or had sensory retina on ILM specimens.

B-2. Postoperative Results

During follow-up period, five eyes (28 %) needed cataract surgery. We indicated phacoemulsification with intraocular lens implantation on the capsular bag. One eye was operated in the first month, two (11 %) at 6th month and another two (11 %) at 9th month. The mean ± SD of VA was 0.153 ± 0.082 before surgery, 0.157 ± 0.114 at one month, 0.195 ± 0.138 at three month, 0.225 ± 0.162 at six month and 0.287 ± 0.233 at twelve month of follow-up. VA progression during follow-up period was showed in Figure 13. Thirteen eyes (72 %) had improved the VA, two (11 %) had no change, and three (17 %) had worsened the VA, at the end of the follow-up.

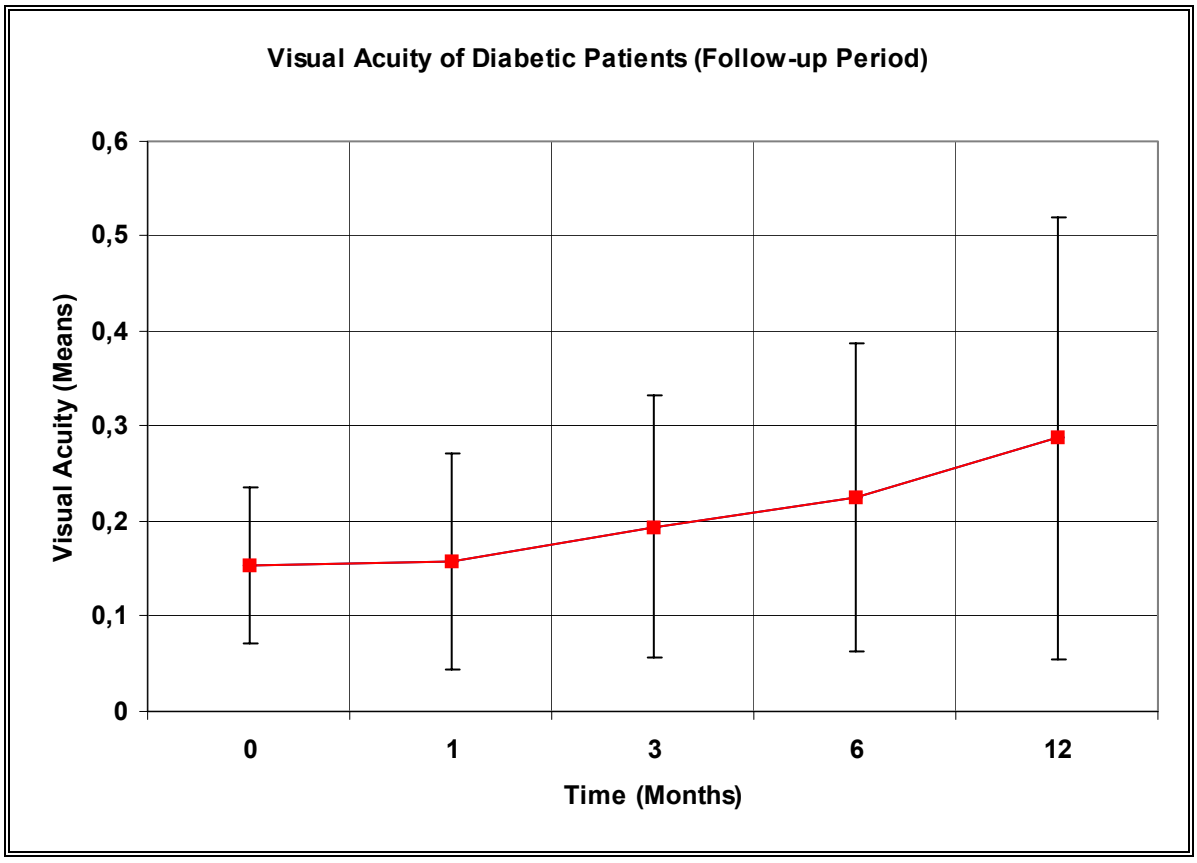


Figure 13. VA after surgery in DME group. Improvement in VA was observed in 14 patients after surgery. The mean ± SD of VA was showed.

With the objective to analyse and demonstrate the progression of VA, the statistical analysis evidenced that the mean of postoperative VA at six month of follow-up was significantly better than those registered in preoperative time ($p = 0.05$). We also observed that the mean postoperative VA at twelve month of follow-up was significantly better than those observed before surgery ($p = 0.02$). Otherwise, the mean of postoperative VA at twelve month of follow-up was significantly better than those at one month after surgery ($p = 0.02$). Thus, the improvement of the visual acuity began to be significant between the third and sixth months after surgery. The significant improvement of VA six month after surgery could be observed in Figure 14.

In the patients 3, 11 and 17, the histological analysis of the ILM, showed the presence of neuroretinal elements in the samples examined by TEM. Those elements were on the retinal side of the ILM and were always accomplished with other cellular elements (glial cells, macrophages, epithelioid-like cells) (see Figure 26). Only the patient 3 had a better best-corrected VA at the end of follow-up. By using FA we concluded that the macular ischemia in patients 15 and 16 was worsened at the end of follow-up. In those patients, we could not collect ILM for histological evaluation during surgical approach.

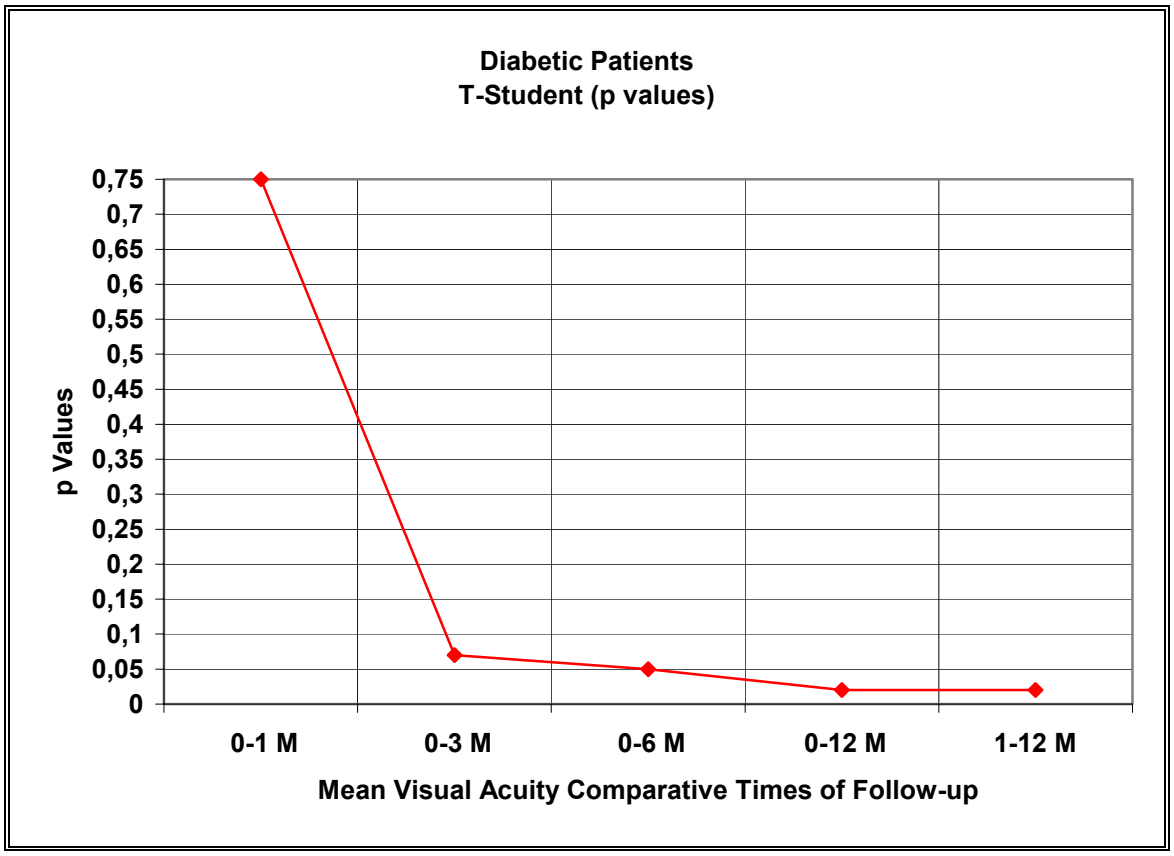


Figure 14. VA recovery in DME group. Statistical significant improvement of VA was evident after sixth month follow-up.

B-3. OCT Results

By using OCT, the mean ± SD foveal thickness was 533.2 ± 132.9 μm before surgery, 402.1 ± 136.2 μm at one month, 375.7 ± 155.5 μm at three month, 355.9 ± 162.6 μm at six month and 292.9 ± 118.4 μm at twelve month after surgery. The improvement of foveal thickness after surgery in DME eyes was represented in Figure 15. We could demonstrate that the gradual diminution observed in foveal thickness was due to the absorption of subretinal and intraretinal fluid, collected in macular area (Figure 16).

At the end of study, only one eye (6 %) had a thickened fovea (424 μm), compared with the preoperative value (417 μm). The others two patients who worsened the VA during the follow-up, presented an improvement of the initial foveal thickness but the histological analysis of the ILM, demonstrated the presence of neuroretina on it retinal face.

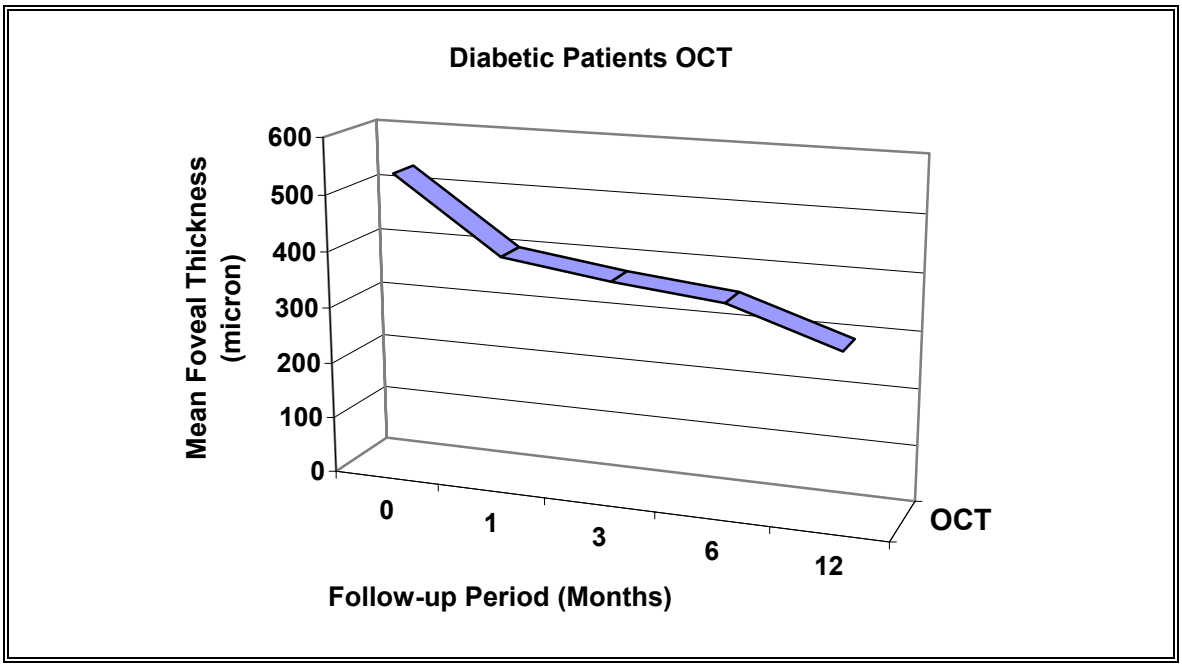


Figure 15. Evolution of foveal thickness after surgery. Along the study and using OCT foveal thickness examination we could observe the regression of thickened maculas.

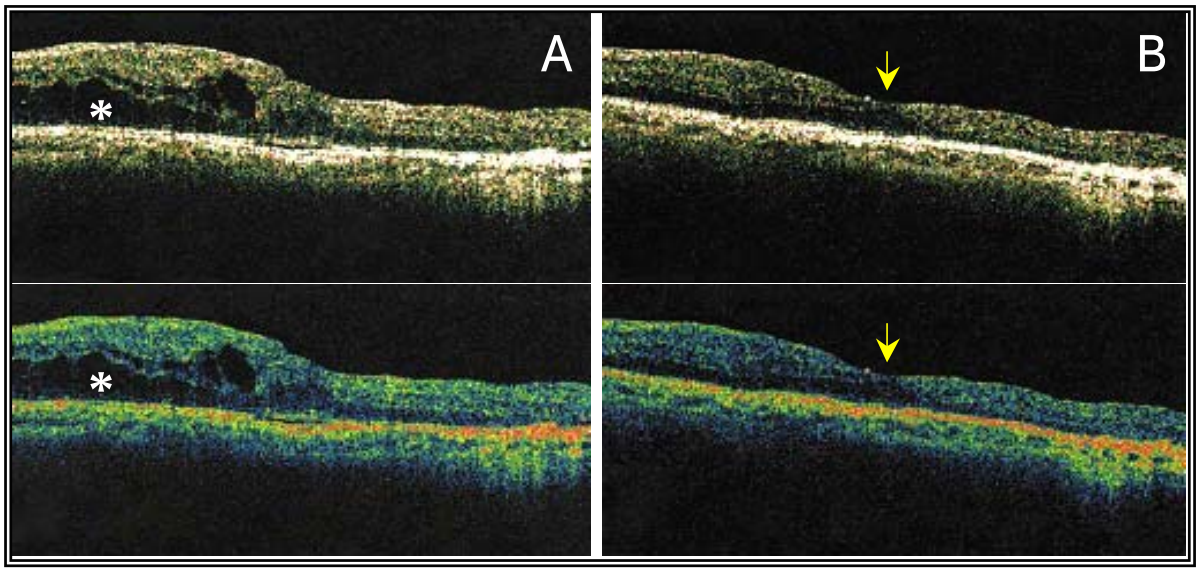


Figure 16 - OCT analysis of DME after surgery. The postoperative OCT realised at first month after surgery on the diabetic eye presented in Figure 9, showed sub-retinal and intra-retinal fluid accumulation in foveal area (asterisks). Twelve months after surgery (B) we could observe the partial resolution of macular edema with acceptable pattern of foveal depression (arrows). OCT foveal image of the same eye (B), showed a thinnest fovea (arrows) at the end of follow-up.

We also could demonstrate that the postoperative foveal thickness was always significantly and progressively thinner than those observed before surgery. The diminution of macular edema demonstrated by the foveal thickness analysis on OCT during follow-up, was showed in Table VI.

Table VI. Foveal thickness evolution during follow-up in DME group

Mean ± SD foveal thickness	Comparative Times of Means Foveal Thickness Follow-up	p Value (*t* student test)
533.2 ± 132.9 µm (0 month)	-	-
402.1 ± 136.2 µm (1 month)	0-1 M	0.00016
375.7 ± 155.5 µm (3 month)	0-3 M	0.00012
355.9 ± 162.6 µm (6 month)	0-6 M	0.000036
292.9 ± 118.4 µm (12 month)	0-12 M	0.000028

Futhermore we observed an evident and graded diminution of the macular thickness in parallel with the progressive improvement of VA along the one-year follow-up. This correlation was very significant since the beginning until the end of the study. Thus, the thinner the fovea was (OCT measurements) the better the mean best-corrected VA. Those results, was represented in Figure 17.

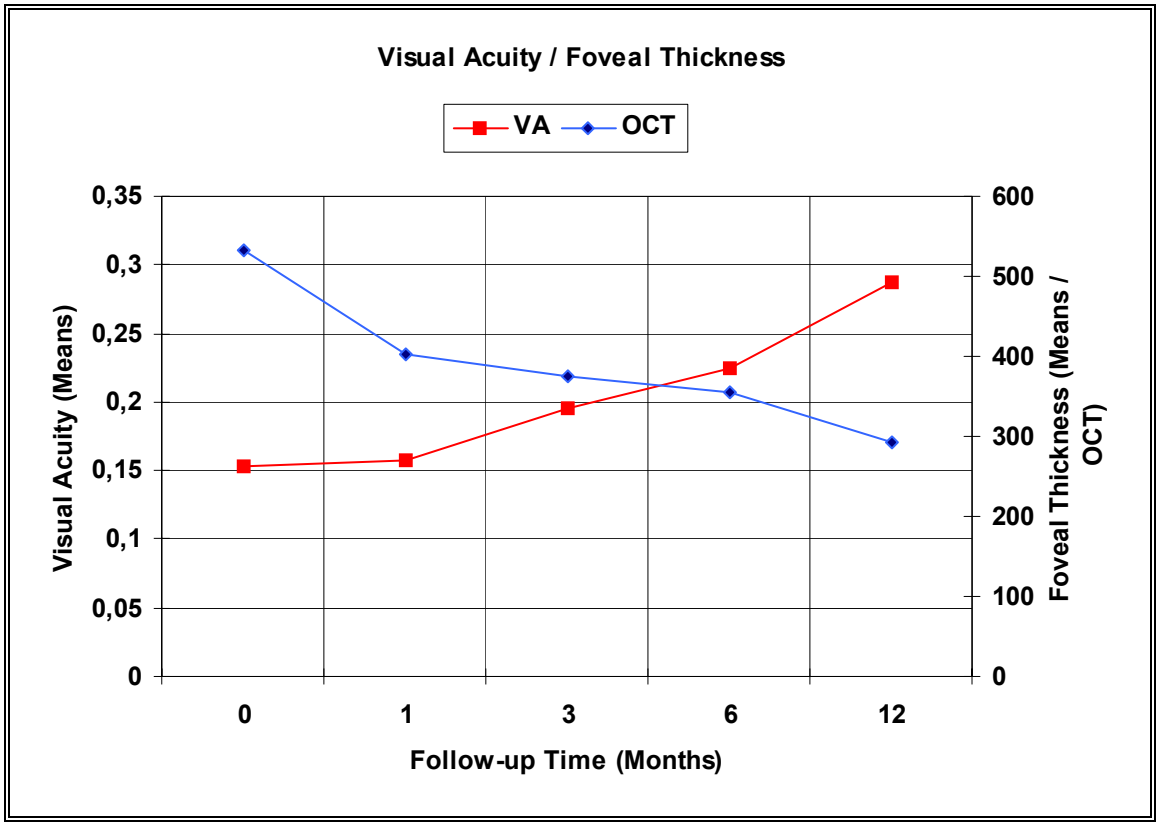


Figure 17. VA and foveal thickness relationship observed in DME group. OCT allows establishing a correlation between VA and foveal thickness. We could observe that the thinner the fovea was the better the mean best-corrected VA.

C. Histopathological Results

In the present study, we studied the ultra-structural characteristics of the normal ILM in retinas obtained from two donated eyes for corneal transplantation and compared to ILM obtained from eyes submitted to PPV. We carried out a histopathological analysis by using light microscopy (LM) and transmission electron microscopy (TEM) in 10 specimens from MH group and 10 from DME group.
ILM specimens were studied regarding its aspect, morphology, uniformity, structure, variability and thickness. Another objective of this approach was also the evaluation of the extra-cellular material and cellular component associated to ILM on both sides, retinal and vitreous face. In pathologic groups we tried to observe and analyse ultra-structural differences in ILM.

C-1. Normal Retina

As a control group we performed LM and TEM studies in normal retinas. Semi-thin sections for LM and ultra-thin sections for TEM were obtained at the level of periphery, equator and macular retinal areas to establish the differences in histological characteristics and findings of ILM regarding these three different anatomic localizations.
In TEM images of the normal retinas, the sections photographed were projected and 75 measurements of ILM thickness were obtained. For each retinal region 5 measurements for each microphotography (n = 5) were performed by random superposition of an unbiased test-volume frame (4,23 mm^2 / test point). A total of 25 measurements of each anatomical retinal area were obtained and then the mean ± SD was calculated. We contacted that ILM was thicker in macular area than the equator and periphery of the retina. Moreover, ILM on equatorial retina was thicker than observed in peripheral areas (Table VII). These differences were very significant (Table VII).

Table VII. Mean ILM thickness in normal eyes, measured in different regions of the globe: periphery, equator and macula.

ILM Thickness (Mean ± SD)	**Macula**	1.35 ± 0.35 µm
	Equator	0.79 ± 0.0 µm
	Periphery	0.30 ± 0.04 µm
Statistical signification (Two tailed *t*-student test)	**Equator / Periphery**	p = 0.000133
	Macula / Equator	p = 5.4821E-06
	Macula / Periphery	p = 4.604E-20

The ILM was thicker in macular area, and very thin in the periphery. We also showed the statistical signification of the differences achieved in ILM thickness measurements, between regions (macula, equator and periphery) of the retina in normal eyes. Histological aspects of ILM observed in normal eyes at different anatomical regions by using LM and TEM were showed in Figure 18.

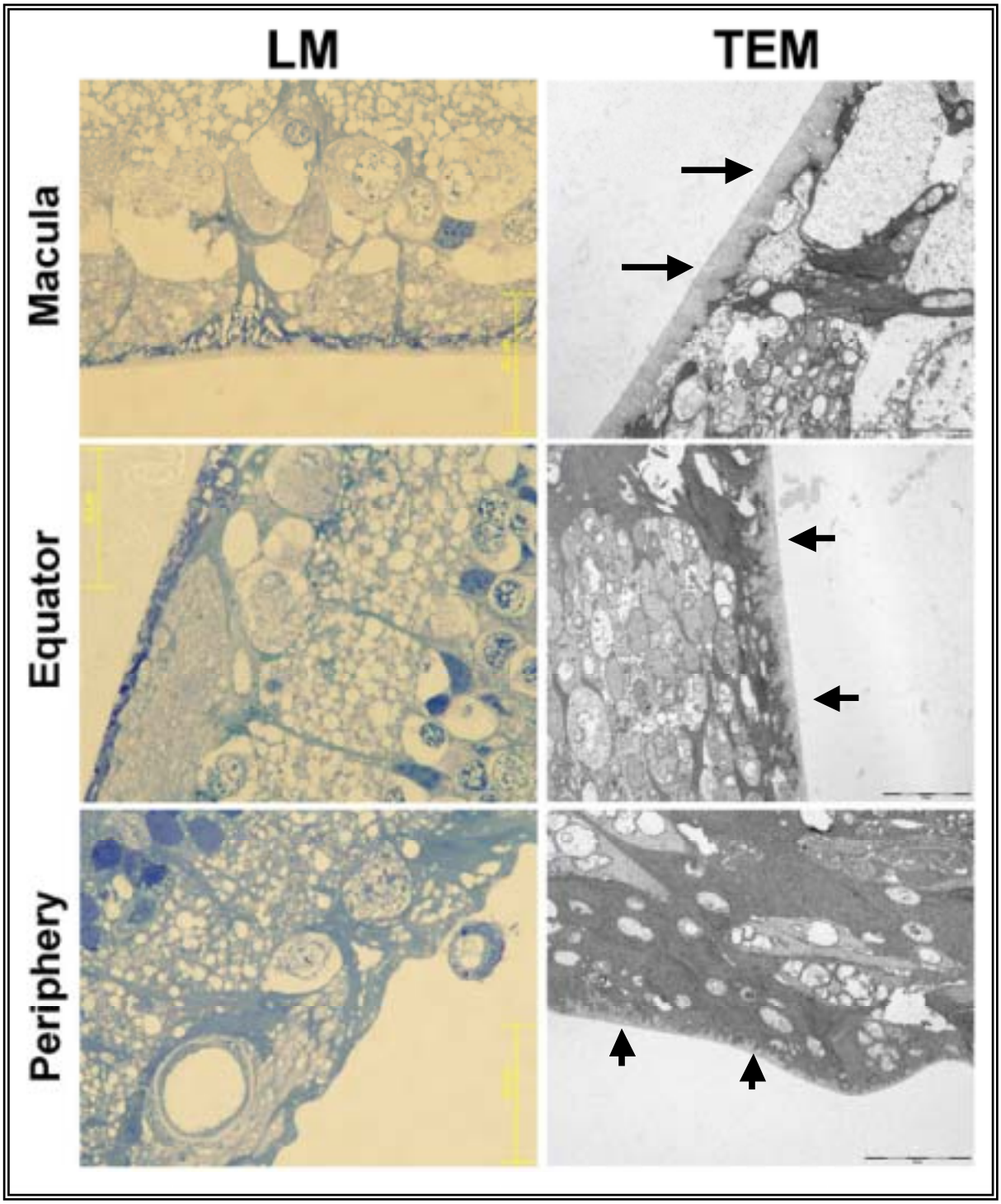


Figure 18 - Light microscopy (LM) and transmission electron microscopy (TEM) of human normal retina. ILM at different levels of topographic retina could be observed and measured. ILM (arrows) is thicker in macula than equator or periphery. Bar LM: 25 μm; Bar TEM: 5 μm.

C-2. Macular Hole Group

We carried out the histopathological evaluation in 10 specimens obtained and prepared from MH group.

We could observe in MH specimens examined by LM and TEM the presence of ILM in 9 of 10. Often large segments of ILM were present in sections (Figure 19). In almost of them, ILM looks irregular, sometimes degenerated and others presenting alterations in its thickness (Figure 19A, B, C and Figure 20). Several segments of ILM presented disruption in the integrity of structural laminas (compare Figure 2 with Figure 20). In 4 ILM specimens we could observed the presence of scanty amount of collagen fibers resembling initial epiretinal membrane (ERM) formation or pieces of thickened posterior hyaloid (Figure 19D and Figure 23).

It is important to stand out that we found remnants of neurosensory retina associated with ILM retinal face in 3 specimens (Figures 19C, Figure 20A and Figure 22B). This histopathological finding could be confirmed by LM and TEM observation.

In one specimen obtained from MH group there was no ILM fragments (Figure 21). This piece presented moderate amount of extra-cellular matrix with an irregular distribution. LM observation revealed an abundant cellular component, which was constituted of several cell types organized in clusters (Figure 21B and C). Pigmented cells displayed epithelioid-like morphology (Figure 21C), cells with abundant cytoplasm with regular clear nucleus resembling glial-like cells (Figure 21B and C) and some cells with fibroblastic morphology (Figure 21B) could be identified. This tissue also showed few capillaries in it periphery (Figure 21D).

In almost all ILM the retinal face was tortuous and irregular and the vitreous face presented a smooth surface (Figure 19, Figure 20A and Figure 22A). Small number of ILM presented a cellular component associated with it vitreous face (Figure 22 and Figure 23A). Most of these cells showed fibroblast-like morphology (Figure 22A) or presented abundant cytoplasm with regular nucleus, which revealed condensate chromatin (Figure 22B). Those cells seem glial-like cells.

Finally, in some ILM evaluated we could observe the presence of posterior hyaloid condensed fragments sometimes associated with cellular component (Figure 23).

The prevalence of main histopathological findings observed in MH tissue extracted during surgery, was showed in Table VIII.

Table VIII. Histological morphology and variability of macular hole ILMs.

	GC	EC	FC	M	Cap	NR	Col	ILM
LM	30 %	20 %	20 %	20 %	20 %	30 %	40 %	80 %
TEM	40 %	-	-	10 %	10 %	30 %	30 %	90 %

LM: light microscopy; TEM: transmission electron microscopy.
GC: glial cell; EC: epithelioid-like cell, with or without pigment; FC: fibroblast-like cells; M: macrophages.
Cap: capillaries; NR: neurosensory retina; Col: collagen fibers; ILM: internal limiting membrane.

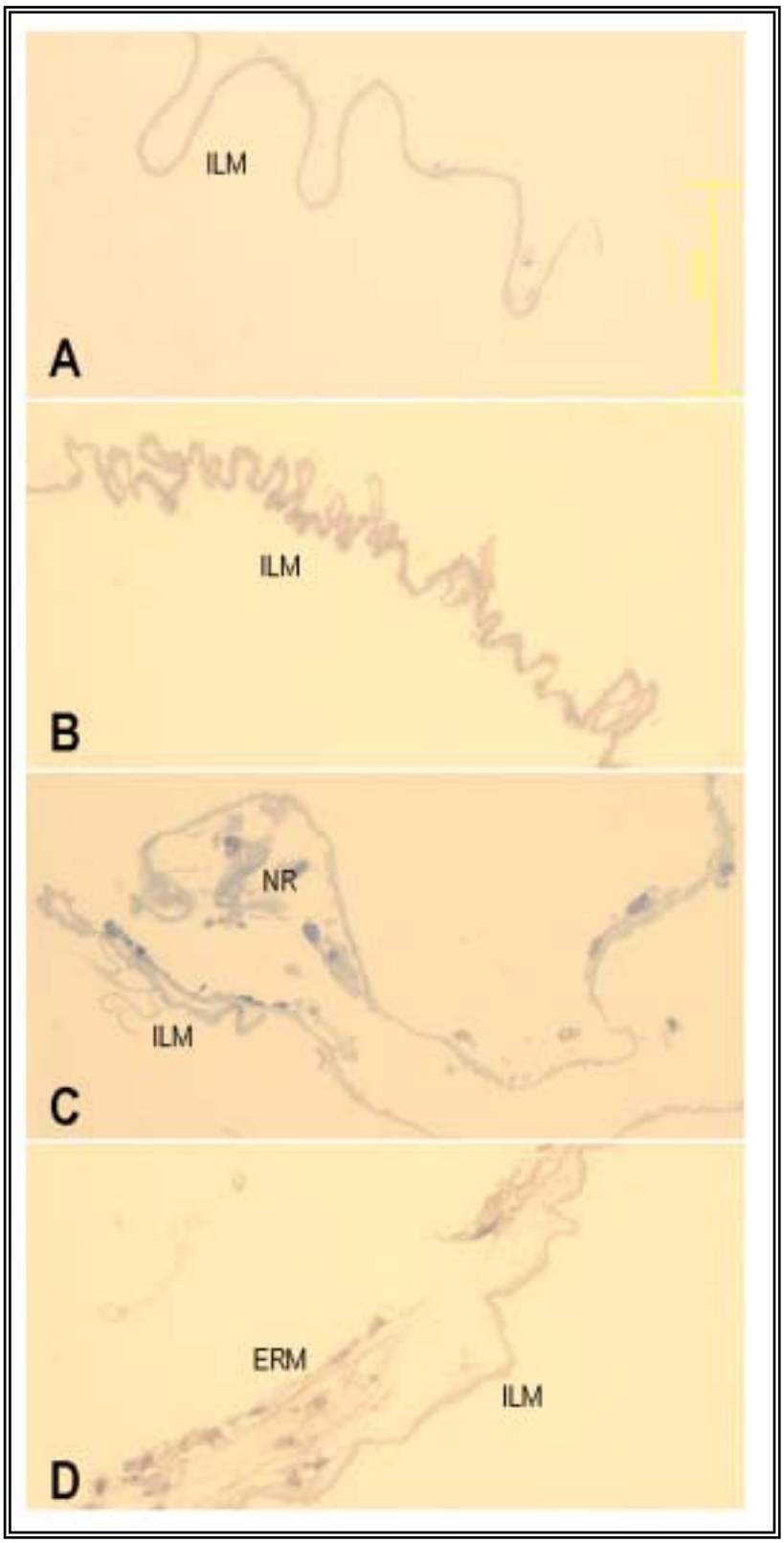


Figure 19 – Semi-thin sections for LM of ILM and epiretinal membranes (ERM) extracted during PPV, in MH group. (A) Intact segments of ILM were observed; (B) Some specimens revealed irregularities or degeneration in ILM structure. (C) Some specimens were associated with remnants of neurosensory retina in ILM retinal face. (D) ERM was also observed associated with scanty fibrillar extra-cellular matrix component. Bar for A - D: 63 μm.

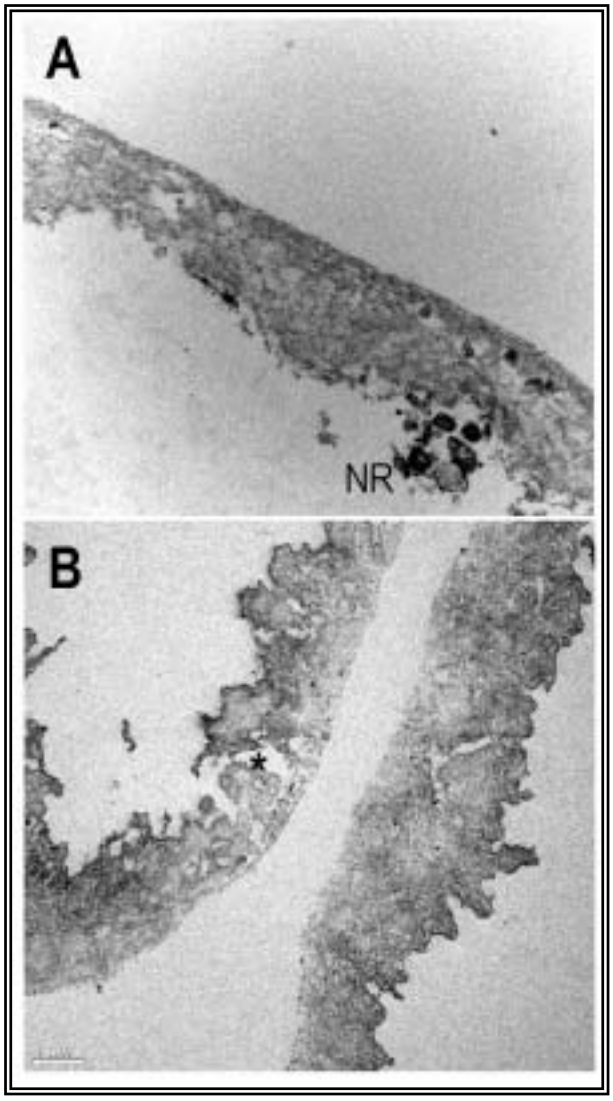
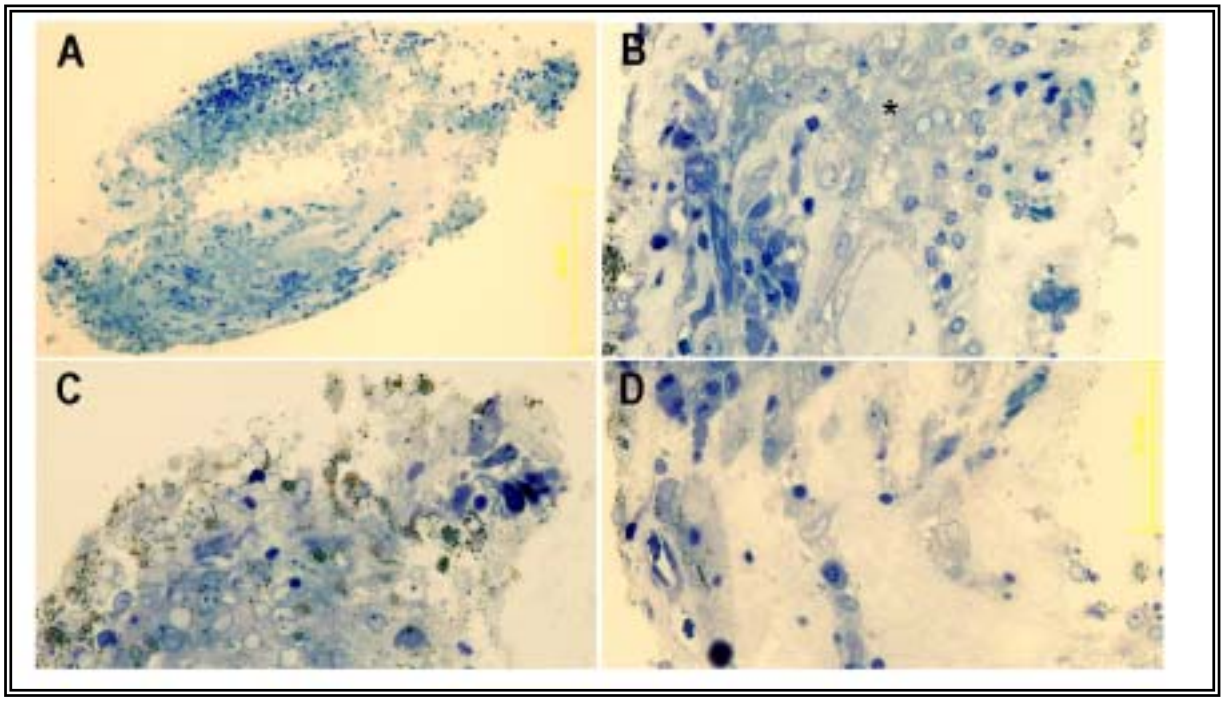


Figure 20 – Ultra-thin sections for TEM of ILM and extracted during PPV, in MH group. (A) Several specimens showed it retinal face tortuous and irregular and it vitreous face with a smooth surface. Neurosensory retina (NR) remnants could be observed associated with some ILM. (B) We could also observed disruptions in the integrity of structural laminas (asterisk) that composed ILM. Bar for A and B: 1 μm.

Figure 21 – Semi-thin sections for LM, of MH tissue extracted during PPV. (A) In this MH extracted tissue there was no ILM; (B) Abundant cellular component was constituted by clusters of glial-like cells (asterisk) and cells with fibroblastic characteristics; (C) Some clusters were composed by epithelioid-like cells presenting or not great amount of intracellular pigment; (D) This specimen showed abundant fibrillar extra-cellular matrix with few small capillaries situated in it periphery. Bar for A: 251 μm; Bar for B, C and D: 63 μm.



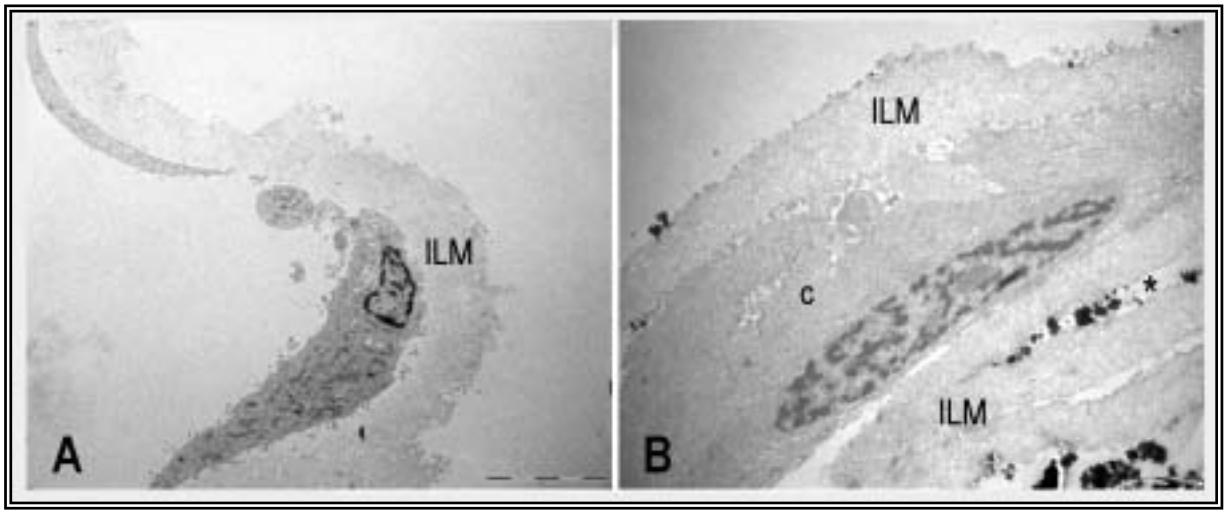


Figure 22 – Ultra-thin sections for TEM of ILM and extracted during PPV, in MH group. (A) Intact fragment of ILM with fibroblast-like cell adherent on it vitreous smooth face. (B) In some specimens, cellular component were composed by glial-like cells that revealed condensate chromatin (c). Remnants of neurosensory retina could also be seemed in the irregular retinal face of ILM (asterisk). Bar for A: 5 μm; Bar for B: 2μm.

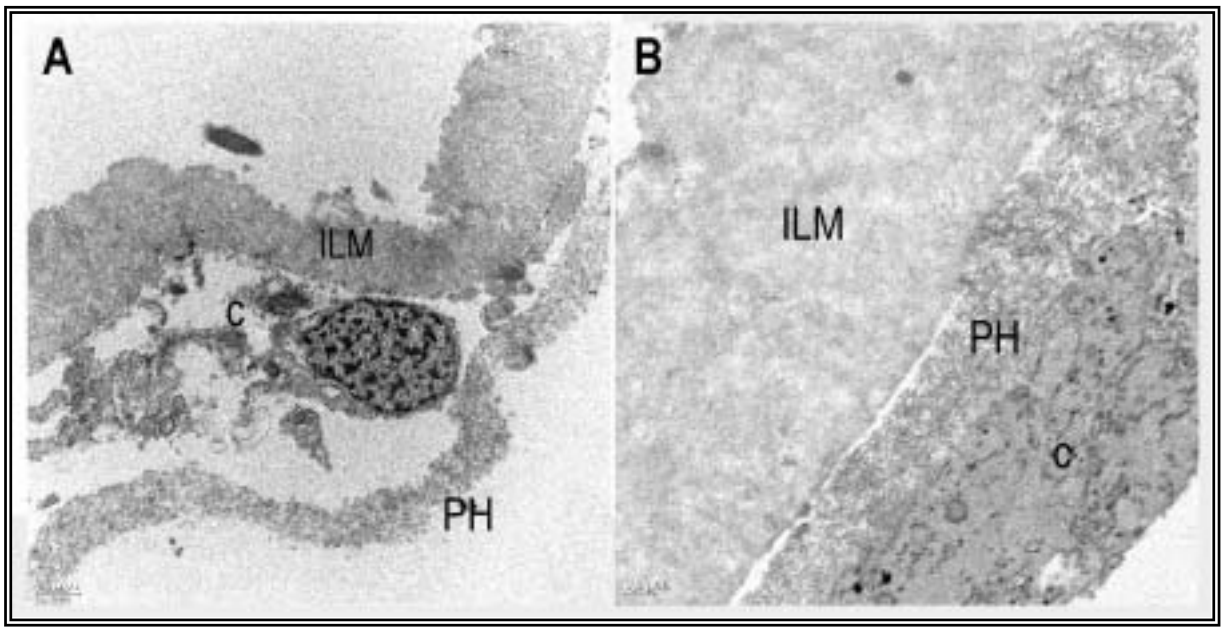


Figure 23 – Ultra-thin sections for TEM of ILM and extracted during PPV, in MH group. (A) Intact fragment of ILM with degenerated glial-like cell (c) situated between it vitreous smooth face and densified posterior hyaloid (PH). (B) Thickened posterior hyaloid (PH) associated with cellular components adherent on the ILM vitreous face. Bar for A: 1 μm; Bar for B: 0.2 μm.

To evaluate the thickness of ILM specimens extracted from MH group we carried out 75 measurements corresponding to several segments of ILM and compared to data obtained from normal macular pieces. Results showed that the mean of ILM thickness in MH group (2.5 ± 1.31 µm) was bigger than those observed in normal maculas (1.35 ± 0.35 µm): this difference was very significant (p = 0.00011). Furthermore when we compared the mean of thickness observed in MH group with those obtained in DME group (2.2 ± 0.78 µm) the difference was also significant (p = 0.03). These conclusions could be seen in Table IX.

Table IX. Stereological analysis on ILM. Mean ILM thickness in normal eyes, measured in different regions of the globe: macula, equator and periphery. We also showed, the ILM thickness in the problem groups and the statistical differences analysed between groups.

NORMAL EYES ILM Thickness (Mean ± SD)	**Macula**	1.35 ± 0.35 µm
	Equator	0.79 ± 0.0 µm
	Periphery	0.30 ± 0.04 µm
PATHOLOGIC EYES ILM Thickness (Mean ± SD)	**MH Group**	2.5 ± 1.31 µm
	DME Group	2.2 ± 0.78 µm
Statistical signification (Two tailed *t*-student test)	**MH / DME**	p = 0.0364
	MH / Normal macula	p = 0.000110
	DME / Normal macula	p = 0.00003618

C-3. Diabetic Macular Edema Group

From 10 specimens obtained and prepared from DME group we could only carried out an accurate histopathological analysis in 8 cases. Two specimens presented generalized structural degeneration probably by inadequate fixative process. In those we only try to identify the presence of ILM in specimens.

In diabetic specimens examined by TEM we could observe the presence of ILM in 7 of 10. When the ILM was present in sections, sometimes it looks normal and uniform but in another areas of the pieces it looks thin and degenerated (Figure 25A and B) Otherwise, in 3 of 10 there was no ILM and an epiretinal membrane (ERM) was manifest presenting a large amount of extra-cellular matrix resembling collagen (Figure 25C and Figure 27) and a great variability of cells (Figure 24 and Figure

25C). Small number of ILM was surrounded with cellular debris on its retinal face. In almost all ILM the retinal face was tortuous and irregular and the vitreous face was smooth (Figure 2, Figure 24A, Figure 25 A and B).

Segments of ILM without cellular or fibrillar components (Figure 24A, Figure 25A and C) was the only histopathological finding observed in 5 specimens by TEM. Other ILM specimen was accomplished with cellular component in it vitreous face, resembling glial-like cell type (Figure 24F). Four specimens presented abundant collagen arrangement compatible with thicken hyaloid or ERM associated or not with segments of ILM (Figure 24B, Figure 25C).

Several types that surround the extra-cellular collagen composed cellular population associated to diabetic tissue. TEM and LM examination showed that these cells were mainly: a) glial-like cells (Figure 24 and Figure 26B); b) fibroblastic-like cells (Figure 24D and Figure 25C); c) epithelioid cells with or without pigment (Figure 24D and Figure 26A); d) macrophages (Figure 26C) and e) plasmatic cells. In 2 diabetic specimens we could observe small capillaries that presented a well-defined basement membrane. Capillaries were always surrounded by abundant collagen fibers deposition (Figure 27).

By TEM examination we could demonstrate the presence of neurosensory retinal components associated with the retinal face of ILM in 3 specimens (Figure 25D)

The prevalence of main histopathological findings observed in diabetic tissue extracted during surgery, was showed in Table X.

Table X. Histopatological morphology and variability analysed in diabetic tissue.

	GC	EC	FC	M	Cap	NR	Col	ILM
LM	37,5 %	37,5 %	25 %	25 %	25 %	25 %	37,5 %	62,5 %
TEM	25 %	25 %	-	12,5 %	12,5 %	37,5 %	37,5 %	70 %

LM: light microscopy; TEM: transmission electron microscopy.

GC: glial cell; EC: epithelioid-like cell, with or without pigment; FC: fibroblast-like cells; M: macrophages.

Cap: capillaries; NR: neurosensory retina; Col: collagen fibers; ILM: internal limiting membrane.

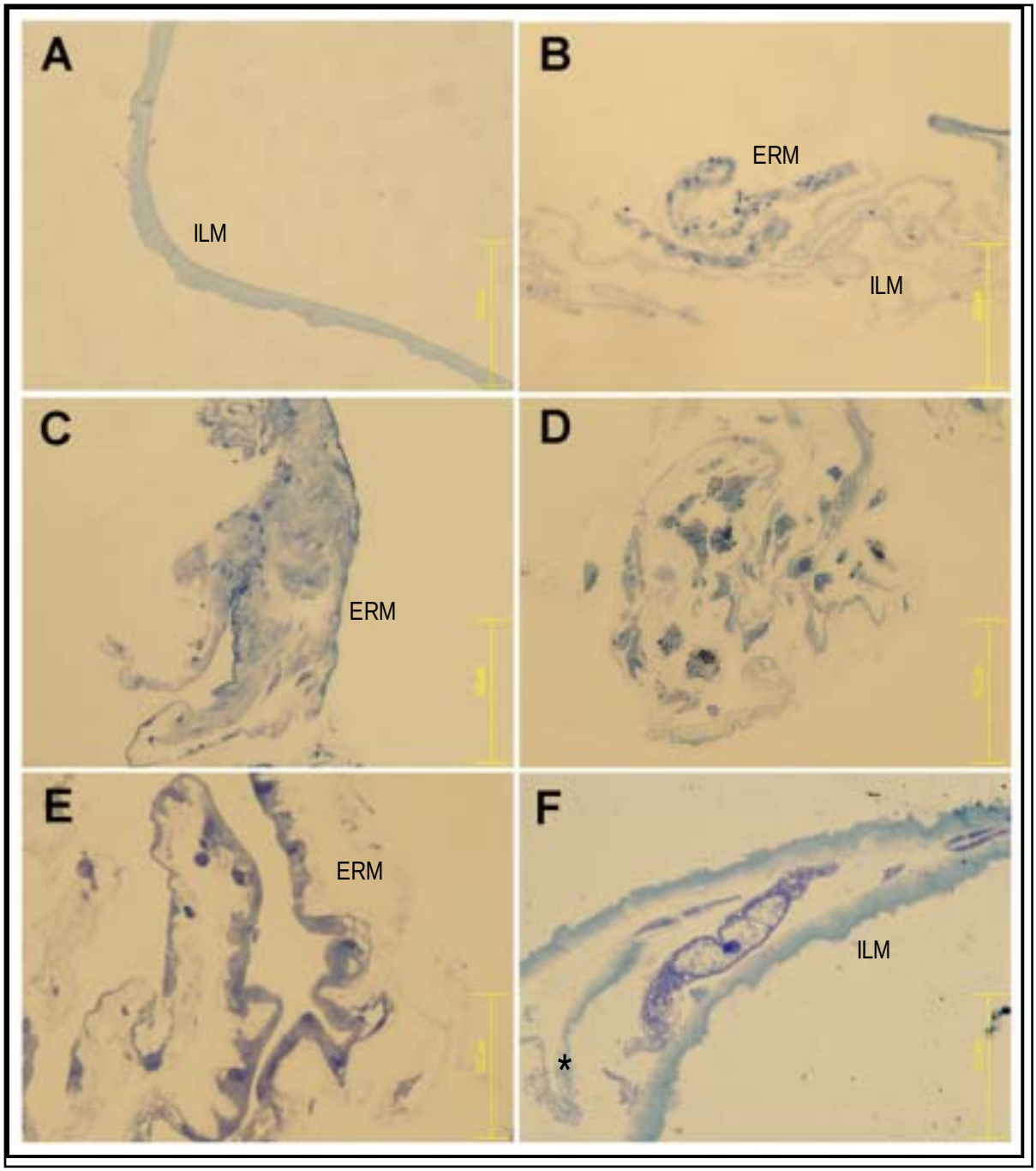


Figure 24 – Semi-thin sections for LM of ILM and epiretinal membranes (ERM) extracted during PPV, in DME group. (A) Intact segments of ILM; (B) In some specimens ERM was associated with large fragments of conserved and altered ILM. (C) Some specimens were constituted only by ERM. (D) ERM was associated with important cellular component in which could be seen fibroblastic-like and epithelioid-like cells and macrophages. (E) ERM was also associated with abundant extra-cellular component that serves to cell adhesion and migrates. (F) Some fragments of ILM were degenerated with diminution of thickness (asterisk). Bar for A and F: 25 μm; Bar for D and E: 63 μm; Bar for B and C: 125 μm.

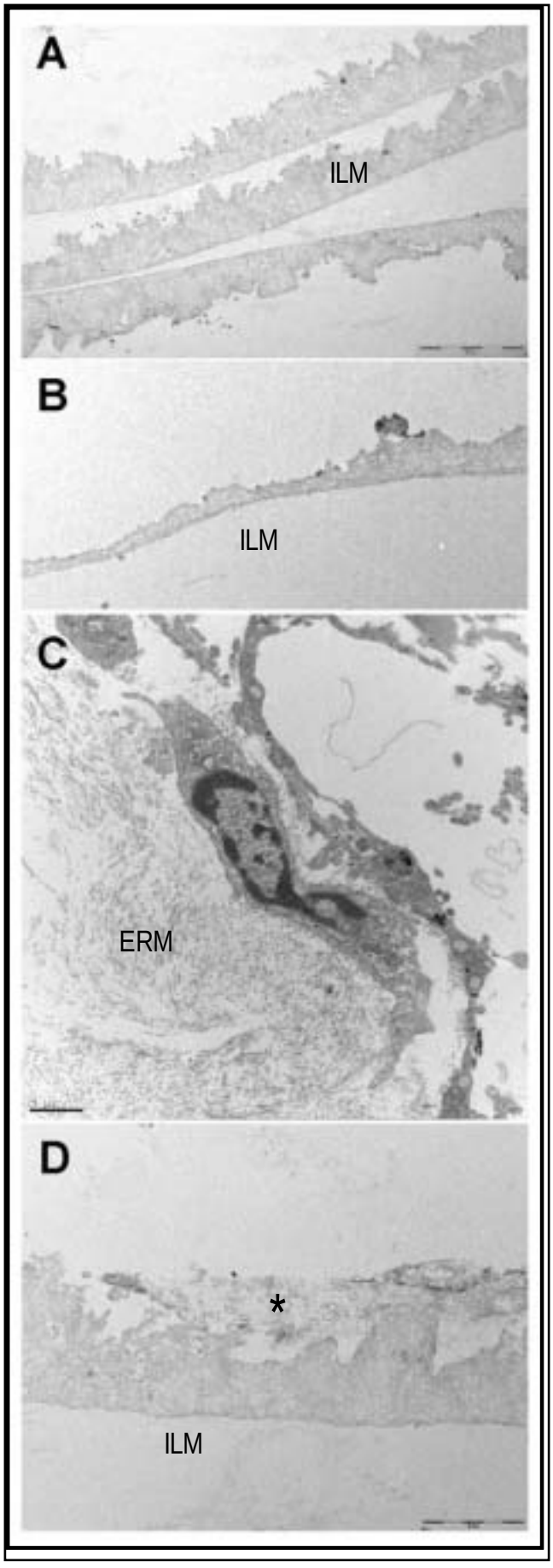


Figure 25 – Ultra-thin sections for TEM of ILM and ERM extracted during PPV, in DME group. (A) Intact fragments of ILM. (B) Decrease of thickness in altered fragment of ILM. (C) ERM was constituted by extra-cellular (collagen) and cellular component. Fibroblast-like cell could be observed. (D) Cell debris of neurosensory retina (asterisk) was sometimes associated with retinal face of ILM. Bar for A and B: 5 μm; Bar for C: 1μm; Bar for D: 2 μm.

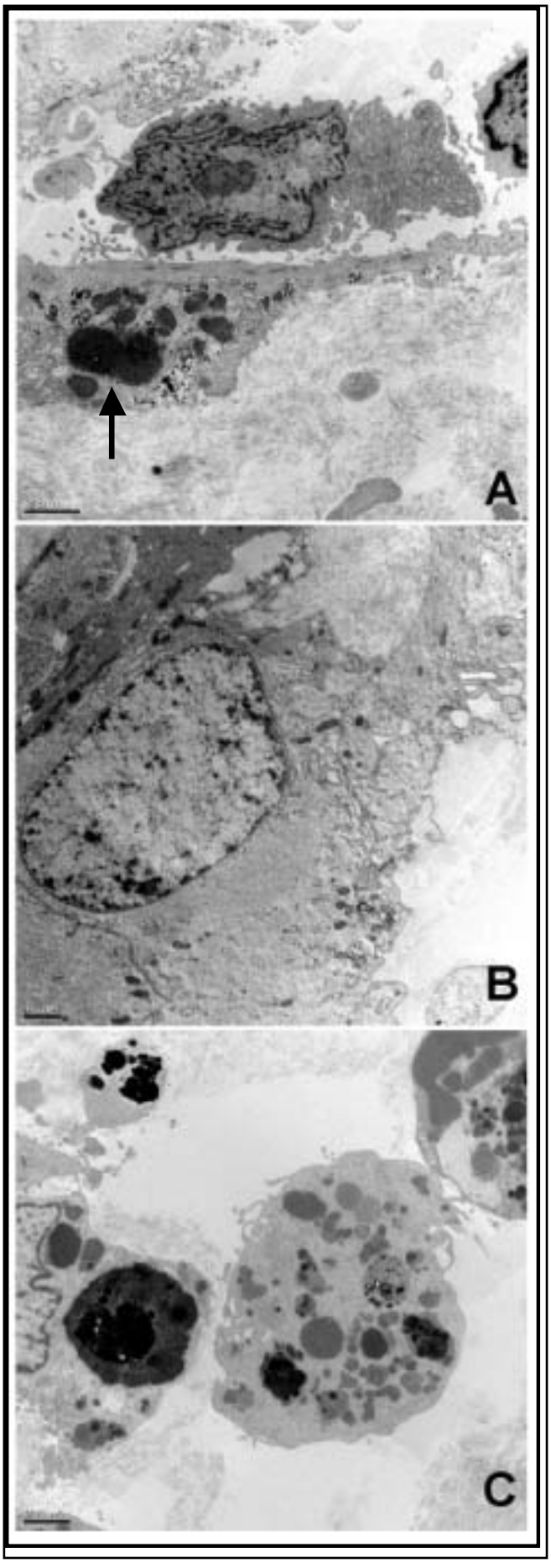


Figure 26 – Ultra-thin sections for TEM of ILM and ERM extracted during PPV, in DME eyes. ILM and ERM cellular component were mainly formed by (A) epithelioid-like cells with or without pigment; (B) Cells with glial characteristics; and (C) Macrophages that presented intra-lisosomal material with different degrees of degradation. Bar for A and C: 2 μm; Bar for B: 1μm.

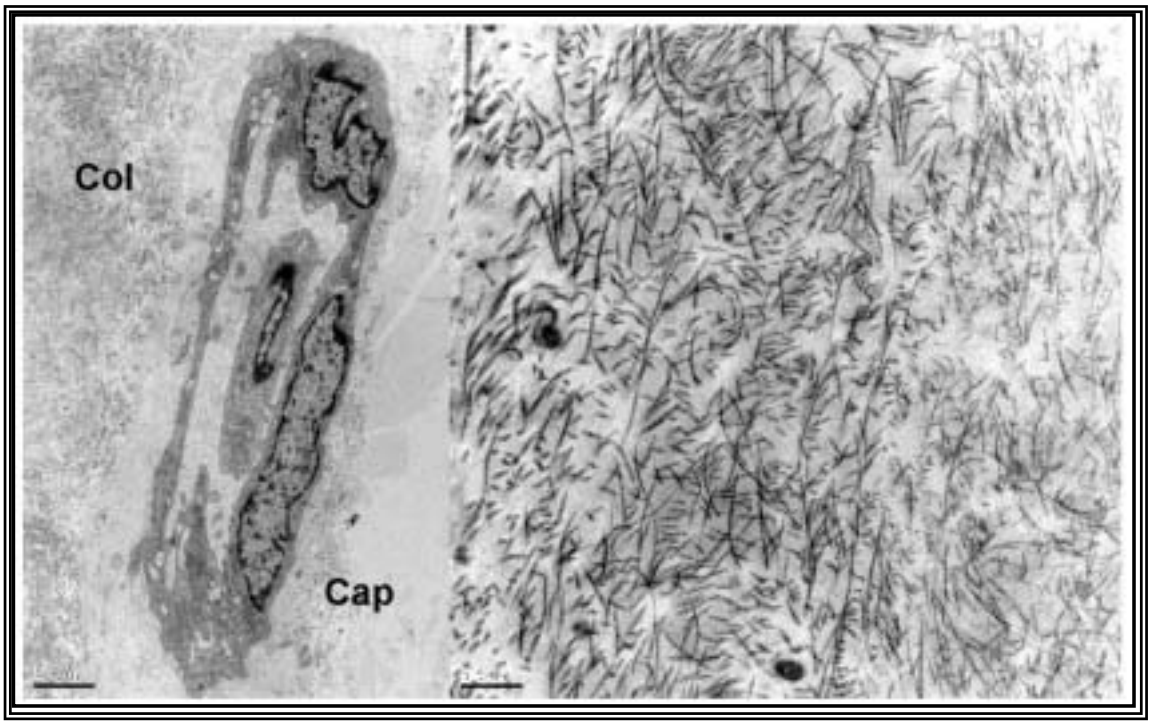


Figure 27 – Ultra-thin sections for TEM of ILM and ERM extracted during PPV, in DME eyes. (A) Some specimens were also formed by small capillaries (cap) surrounded by abundant collagen fibers (col) deposition. (B) Smooth collagen fibers were diffusely arranged and do not presented fibber periodicity. Bar for A: 2 μm; Bar for B: 0,5 μm.

To give insight to the ILM behaviour in DME we try to find differences in thickness between normal and diabetic ILM specimens. For this purpose we carried out 75 measurements corresponding to several segments of ILM obtained from DME group and compared to data obtained from normal macular ILM. Results showed that the mean of ILM thickness in DME group (2.2 ± 0.78 μm) was great than those observed in normal retinas (1.35 ± 0.35 μm). This difference was very significant (p = 3.6E-05). This conclusion could be seen in Table IX.

VI. DISCUSSION

A – Macular Hole Surgery

Although various pathogenic mechanisms have been suggested to cause idiopathic macular holes, there is now a general consensus that direct antero-posterior or tangential traction, or both, via a pathologically altered vitreomacular interface, results in the formation of macular holes (Gass et al, 1988, Gass et al, 1995; Johnson et al, 1988; Smiddy et al, 1989). For this reason, the surgical removal (or peeling) of the ILM has been introduced as a potentially useful method in MH surgery (Park et al, 1999; Olsen et al, 1998, Eckardt et al, 1997).

To peel or not to peel the ILM has been the debate in current MH surgery literature. In evaluating the success of MH surgery, both the primary and ultimate closure rate, the rate of recurrence, and ultimate visual acuity must all be considered. Proponents of ILM peeling have shown in several studies an improved hole closure rate with the peeling, either with or without indocyanine green (ICG). Recently, the visualization of the ILM during surgery was facilitated by the use of ICG, which selectively labels this thin membrane (Burk et al, 2000; Da Mata et al, 2004). Because of this technical improvement, and based on many reports of accelerated closure of macular holes and improved visual acuity in the patients after peeling, the procedure is now generally considered to be a safe manoeuvre (Park et al, 1999; Margherio, 2000; Kadonosomo et al, 2000; Brooks et al, 2000; Da Mata et al, 2004). Nevertheless, the question of “peel or not to peel” is still a matter of debate (Hassan et al, 2002; Smiddy et al, 2001). First, the analysis of some current literature does not tell us if ILM peeling is necessary in MH surgery or if visual outcomes are better with or without peeling (Hassan et al, 2002; Smiddy et al, 2001; Haritoglou et al, 2001; Haritoglou et al, 2002). Second, there were some reasons to assume that damage at the vitreoretinal interface may exert deleterious effects on neuronal function and even survival (Wolf et al, 2004). Wolf and colleagues (2004) saw increasing cellular damage in the transition zone and in the peeled area. Whereas some Müller cells end feet (as well as nearly all axons) remained virtually intact, many Müller cell end feet and their adherent inner processes showed severe swelling or even were extinguished. ILM peeling should be performed with caution or/and may be there was some factors that enhance the adherence between the ILM and the others layers of the retina.

Several works that advocating the use of ILM peeling, reported primary anatomic closure success between 90% and 97% (Park, 1999; Mester et al, 2000; Margherio et al; 2000; Brooks, 2000).

There are three multicentered, controlled, properly conducted, well-designed randomised clinical trials that compare the value of surgery versus observation for MH (Freeman et al, 1997; De Bustros et al, 1994; Kim et al, 1996) (Tables XI and XII). De Bustros et al (1994) (Table XI) reported results in patients with stage 1 macular holes, with very good compliance to protocol and follow-up, but the strict eligibility criteria enrolled a small number of participants, limiting the power of this study to detecting only large treatment effects. Kim and associates (1996) (Table XI), enrolled patients for stage 2 macular holes, with good compliance to follow-up, although there was an unequal allocation to study arms that resulted in fewer eyes than estimated receiving surgery. The study of Freeman et al (1997)

(Table XII) analysed patients with stage 3 and stage 4 macular holes and had very good compliance to protocol and follow-up, although the 6 months follow-up reported was relatively short.

Table XI. Randomised Controlled Trials on Vitrectomy for Early Stages of Macular Holes.

Study	**De Bustros**		**Kim**	
		- Stage 1		**- Stage 2**
Treatment Group	Surgery	Observation	Surgery	Observation
Number Enrolled	27	35	17	25
Follow-up Time (months)	Average 17		12	
Compliance To Follow-up	60/62 (97%)		15/17 (88%)	21/25 (84%)
Progress to Stage 3 or 4	10 (37%)	14 (40%) p = 0.81	3/15 (20%)	15/21 (71%) p = 0.006
>20/40	11 %	14 % p = 0.014		
<20/80	33 %	20 %		

Table XII. Randomised Controlled Trial for Surgery for Stage 3 or 4 Macular Holes.

Study	**Freeman**	
Treatment Group	Original Operation	Observe
Number Enrolled	64	65
Follow-up Time (ms)	6	
Follow-up Compliance	94 %	89 %
Surgical Success	36/52 (69%)	2/56 (4%) (p = 0.001)
Mean ETDRS Score/Vision	0.76 logMAR ; 20/115	0.92 logMAR ; 20/166 (p = 0.05)
Surgical Success Definition	Closure of the hole defined by stereo photos	Clinical

Recently, traditional MH surgery was combined with the use of ICG enhancement for ILM peeling (Sheidow et al, 2003). In those series, both groups undergoing ILM peeling had a significantly improved rate of primary hole closure relative to traditional approaches, with no difference seen

between ILM peeling with or without ICG. Those results compare favourably to all prior studies in the primary closure rate and represent one of the largest comparative series of ILM peeling. This visual result is important because advocating ILM peeling simply on the basis of an increase in primary hole closure would be of little benefit if vision outcomes were not similarly improved. This lower rate of visual improvement in the ICG group opens several questions. ICG significantly enhances the surgeon's view of the ILM, provides a clear visual end point, speeds the learning curve for new surgeons, and ensures that the ILM is removed completely (360º), with a minimal degree of accidental trauma to the retina. This study also found no clinical evidence of retinal toxicity from ICG use. Another possibility is that the use of ICG does result in some toxicity (Sippy et al, 2001, Gandorfer et al, 2001; Engelbrecht et al, 2002) to the photoreceptors or retinal pigment epithelium and limits the visual recovery.

In a recent prospective interventional study, Kusuhara and colleagues (2004) found an anatomic success after the first surgical approach in 94,3% of eyes with stage 2 or 3 idiopathic macular holes, submitted to a standard three-port PPV with ICG assisted ILM peeling. In all the patients, a 0.25% concentration of ICG dye was used for ILM staining and didn't cause major retinal toxicity and the surgical treatment of macular holes had good anatomic and clinical results. These findings, was recently corroborated by Simon and associates (2004). More recently, Da Mata (Da Mata et al, 2004) demonstrates excellent anatomic (98% anatomic closure) and visual (96% improved visual acuity) results, in a long-term retrospective follow-up of patients who underwent ICG-assisted ILM peeling for idiopathic MH (stages 2 – 4) repair. In his study, there were no intraoperative or postoperative complications attributed to the use of ICG.

Nevertheless, a few clinical reports attribute complications and poor outcomes to the use of ICG (Haritoglou et al, 2002; Gass et al, 2003; Engelbrecht et al, 2002). The authors suggest that ICG-assisted ILM peeling results in poor visual outcomes, visual field defects, and RPE defects. It is not entirely clear why some authors have seen poor visual acuity results. However, a short duration of follow-up as well as differences in surgical technique may explain some of the discrepancies.

Surgical peeling of the ILM can be technically challenging even for experienced vitreoretinal surgeons. It seems likely that ILM peeling and the intraoperative use of ICG will remain a controversial topic, with its opponents and proponents. Specific difficulties include initiating the ILM peel, visualization of the border between peeled and unpeeled ILM, and determining the extent of the ILM peel. In the current study, retinal ILM peeling assisted with ICG, has been a useful adjunct to vitreoretinal surgery, and has been demonstrated to improve anatomic and visual outcomes after MH surgery.

In the current prospective study of PPV with ICG-assisted ILM peeling of ten macular holes, the mean ± standard deviation best-corrected visual acuity was 0.14 ± 0.08 before surgery, 0.27 ± 0.14 at six month and 0.31 ± 0.18 at twelve month of the follow-up. The mean postoperative best-corrected visual acuity at the month twelve of the follow-up, was significantly better than the mean preoperative best-corrected visual acuity ($p = 0.003$) and all the patients, but two, had an improvement in the best-corrected visual acuity at the end of the study. The recovery of the visual acuity was also significant after three months of the follow-up ($p < 0.03$). At the end of our study, the MH closure was achieved in

90 % (See Figure 28), with one reopening MH at 10th month of follow-up. No complications were attributed to the use of ICG.

Futhermore, in two patients who didn't improved their VA, the histological analysis by TEM showed neuroretinal components on the retinal side of the ILM. As previously described (Haritoglou et al, 2002; Gass et al, 2003) poor visual acuity outcomes was attributed to the use of ICG and has also described the presence of significant amounts of cellular elements on the retinal side of the ILM after ICG-assisted ILM peeling. The etiology of these observed results is unclear, but it is conceivable that the reason for poor visual acuity outcomes and the presence of cellular elements on the retinal face of the ILM might be consecutive of an aggressive ILM peeling (Gass et al, 2003).

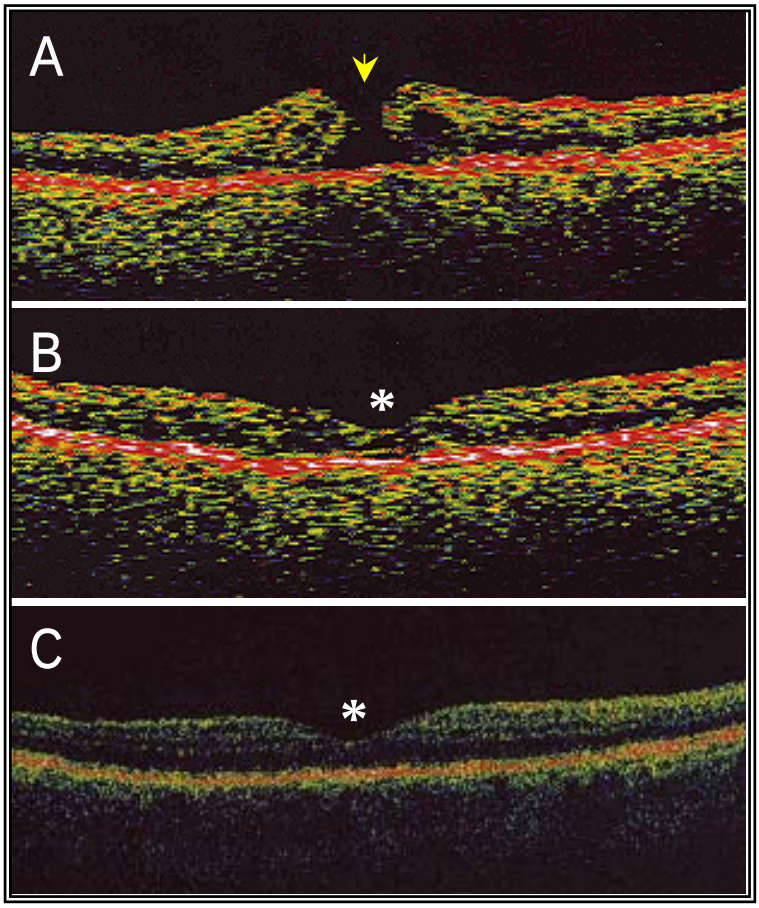


Figure 28 – Macular hole OCT follow-up of the same eye: 0 M (A) stage 4 MH (arrow), 1 M (B) and 6 M (C), showing the normalization of the foveal depression (asterisks), without significant sub-retinal fluid at 6 months.

A-1. OCT and Macular Hole Surgery

Quantitative information (Hee et al, 1995) could be extracted directly from the OCT images, including the diameter of the hole and the extent of surrounding subretinal fluid accumulation. The high resolution obtained in the tomographs suggests that OCT may be a useful method of precisely

monitoring hole progression or recovery after surgery. OCT may be an important tool (Hee et al, 1995) of assessing the vitreoretinal interface and evaluating the risk of hole formation in the fellow eyes of patients with a unilateral MH. The high longitudinal resolution of OCT is more accurate than echography or biomicroscopic examination in evaluating small separations between the posterior hyaloid membrane and the neurosensory retina. However, further study is needed to determine the sensitivity of OCT in detecting the weak reflection from the posterior hyaloid.

Preoperative OCT measurements seem to be of predictive value for the functional and anatomical outcome of MH surgery. In a prospective study, Ullrich and colleagues (2002) concluded that preoperative measurement of MH size with OCT provided a prognostic factor for postoperative visual outcome and anatomical success rate of MH surgery. Similar results, was been published by Freeman and co-workers (1997), who found that a MH with a small diameter was associated with better functional outcome. The reason for this might be that a small hole diameter indicates a better-preserved macula. The most favourable explanation for the development of a MH is traction caused by focal shrinkage of the prefoveal vitreous (Margherio et al, 1972). Also glial cells and newly formed collagen may play an important part in MH formation by exerting tangential traction (Messmer et al, 1998). The diameter of the hole therefore may depend mainly on traction forces and not on the duration of the MH. Another reason for a missing correlation between the duration of symptoms and MH size may be the subjective estimation of the duration of symptoms by the patient: a MH may exist a long time before being detected (Ullrich et al, 2002).

It has been well recognized clinically that patients with better preoperative visual acuity, shorter duration (Wendel et al, 1993), earlier stage (Ryan et al, 1994), and smaller MH size (Ulrich et al, 2002; Ip et al, 2002) have better anatomic and visual success rates, although disagreement in at least one study suggests a more complex interaction may be operative (Tilanus et al, 1999). Recently, Kusuhara and colleagues (2004) tested OCT determined parameters, attempting to identify a reproducible, accurate, and ascertainable parameter that might provide prognostic information. They concluded that the ratio of the separation from the RPE to the inner retina divided by the greatest horizontal dimension of the MH at the level of the RPE (b/a ratio or macular hole index – MHI) was the most clinically useful parameter. In their study ratio that exceeding 0.5 presented better visual prognosis.

On the current study, it was our understanding that OCT was a very useful method to confirming the diagnosis, to establish the classification of all macular holes at the beginning of the study and in the analysis of the foveal status before surgery. Futhermore, OCT was important for the following of the sub-retinal fluid absorption along the time and to evaluate the anatomic hole closure evolution after ILM peeling.

B – Diabetic Macular Edema and Macular Surgery

It has been reported that a posterior precortical vitreous pocket exists immediately anterior to the posterior retina and extends to the temporal vascular arcades (Kishi et al, 1990; Kishi et al, 1996). When a PVD is induced during vitrectomy in an eye with posterior precortical vitreous pocket, a part of the vitreous cortex may remain attached to the ILM as a remnant (Kishi et al, 1986). This remnant can serve as a scaffold for proliferative tissues that can then cause traction on the macula and the recurrence of edema. Recently, the removal of the ILM during vitrectomy has been performed with the complete removal of the vitreous cortex to help treat the DME (Gandorfer et al, 2000). The posterior vitreous cortex is attached to the ILM by intermediary macromolecules such as laminin, fibronectin, chondroitin sulphate, and other extra-cellular matrix components. The higher concentration of laminin and fibronectin in the ILM of diabetic and aged eyes (Kohno et al, 1987; Kohno et al, 1987) most likely leads to a stronger adhesion of the vitreous to the retina. This suggest that vitrectomy with an attempt to induce PVD without meticulous peeling of the posterior hyaloid would leave vitreous cortex remnants on the ILM, which might lead to the formation of pucker and the development of macular edema by traction.

Patients with DME combined with a taut and thickened posterior hyaloid assessed by biomicroscopy (Lewis et al, 1992, Van Effenterre et al, 1993, Harbour et al, 1996, Pendergast et al, 2000, Gandorfer et al, 2000) or OCT (Massin et al, 2003), showed benefits with PPV and removal of posterior hyaloids (Table XIII). In these reports, DME decreased or resolved at least in 85% of cases with improvements in visual acuity in 50% to 100% of patients. PPV was also beneficial for DME in cases without a taut posterior hyaloid, demonstrated by OCT (Tachi et al, 1996; Ikeda et al, 1999; Otani et al, 2000; Giovannini et al, 2000; La Heij et al, 2001; Yamamoto et al, 2001), and when the posterior hyaloid was detached from the posterior pole (Ikeda et al, 2000) (Table XIV).

Table XIII. Results of vitrectomy reported in previous studies for eyes with diffuse DME combined with a taut, thickened posterior hyaloid.

Study	Number of Eyes	VA Improvement > 2 lines (%)	Decrease in or Resolution of DME (%)	Follow-up (Months)	Recurrences
Lewis (1992)	10	6 (60)	10 (100)	16	0
Van Effenterre (1993)	22	19 (86)	19 (100)	14	4
Harbour (1996)	7	4 (57)	6 (86)	12	
Gandorfer (2000)	10	10 (100)	10 (100)	16	0
Pendergast (2000)	55	27 (49.1)	52 (94.5)	23.2	3

DME = diabetic macular edema; VA = visual acuity.

Table XIV. Results of vitrectomy reported in previous studies for eyes with diffuse DME not combined with a taut, thickened posterior hyaloid.

Study	Number of Eyes	VA Improvement > 2 lines (%)	Decrease in or Resolution of DME (%)	Follow-up (M)
Tachi (1996)	58	31 (53)	98%	12
Ikeda (1999)	3	2 (75)	100%	16
Otani (2000)	13	5 (38)	61%	6
Ikeda (2000)	5	4 (80)	80%	8
La Heij(2001)	21	10 (47.6)	100%	11

DME = diabetic macular edema; VA = visual acuity; M = months.

Pendergast and associates (2000) demonstrated the role of vitrectomy in eyes with diffuse DME associated with a taut posterior hyaloid. Vitrectomy with stripping of the premacular posterior hyaloid showed improvement in best-corrected visual acuity and in macular edema. They concluded that in these eyes unresponsive to laser therapy, vitrectomy with removal of the posterior hyaloid appears to be beneficial in some cases. Futhermore final best-corrected visual acuity was strong and inversely related with the extension of macular ischemia.

Earlier studies found that a shorter time interval from initial diagnosis of macular edema to vitrectomy may be associated with a better visual outcome (Harbour et al, 1996). This may be attributed to the destructive effect of long-standing macular edema on the retinal layers (Gass, 1998), and/or may be due to a longer duration of traction by the posterior hyaloid causing a shallow detachment of the macula. These facts has been corroborated by La Heij and colleagues (2001) suggesting that early vitrectomy should perhaps be considered as an alternative treatment for patients with DME without evident macular traction from a thickened vitreous membrane. They showed resolution of DME that was no longer visible on microscopic examination three months after vitrectomy. Moreover, the premacular vitreous in diabetic eyes may also contain factors contributing to the persistence of macular edema (La Heij et al, 2001).

Yamamoto and colleagues (2001) demonstrated that vitrectomy is effective in reducing DME, and the outcomes did not depend on the presence or absence of a posterior vitreous detachment or an epimacular membrane. One possible mechanism could be that the preretinal oxygen tension was significantly higher in vitrectomized eyes than in the non-vitrectomized (Stefansson et al, 1990). They suggested that the retina oxygenation increased after vitrectomy because oxygen was supplied from arterial blood in the ciliary's processes. Because high concentrations of oxygen tension cause retinal vasoconstriction (Bill et al, 1981; Deutsch et al, 1983) it could be suggested that the increased levels of oxygen in the vitreous may reduce vascular leakage and, thereafter, may reduce DME.

Massive macular exudates may impair visual acuity severely, and they are refractory to conventional laser treatment (Yang, 2000). Combined PPV with posterior hyaloid removal, and endolaser treatment, was also effective in eyes with massive hard exudates secondary to DME (Yang, 2000). All eyes showed significant decreases in macular edema and hard exudates, a process that became clinically obvious 3 months after surgery.

Gandorfer and colleagues (2000) evaluate the surgical results of PPV with peeling of the ILM from the macula in eyes with diffuse DME. Intra-operatively, the posterior hyaloid was found thickened and completely attached to the macula in most of the eyes. Postoperatively, retinal thickening resolved or decreased and visual acuity improved. No recurrence or deterioration of macular edema or epiretinal membrane formation was observed during the entire period of review. They concluded that the removal of the ILM decreases the risk of subsequent epiretinal membrane formation by eliminating the scaffold for proliferating cells. Moreover, the removal of the ILM theoretically may lead to changes in the cytoskeleton of the retinal cells and thereby enable more rapid resolution of diffuse macular edema (Gandorfer et al, 2000).

In our prospective study of eighteen eyes with DME submitted to PPV with ILM extraction in macular area, the visual acuity improved in 72 % of the eyes and maintained in 11 %. Impairment of visual acuity was observed in 17 %. Fluoresceinic angiography (FA) carried out in these patients revealed worsening of macular ischemia. Unfortunately, the ILM was not collected during the surgical treatment of these patients and no data about histopathological findings could be achieved.

Recent studies have shown that VEGF causes conformational changes of the tight junctions of retinal vascular endothelial cells (Gardner et al, 2002) and plays a major role in increasing vascular permeability in diabetic eyes (Ciulla et al, 2002). In another recent study (Itakura et al, 2004), Itakura and associates concluded that, a higher VEGF level was maintained in the vitreous cavity after vitrectomy for PDR, 10 folds higher than that observed in the plasma. Thus, there is a persistent secretion of VEGF into the vitreous cavity after vitrectomy for PDR. Nevertheless that retinal photocoagulation may reduce the production of VEGF (Aiello et al, 1994), they speculated that those high levels of VEGF after PPV without retinal photocoagulation, may contribute to explain the worsened visual acuity after surgery to treat PDR.

Finally, it has been demonstrated that changes in capillary blood flow velocity were significantly correlated with the improvement in visual acuity (Kadonosono et al, 2000). Increase of perifoveal microcirculation after surgery may be mainly the result of the decreased tissue pressure associated with disappearance of EMD.

In our study, we showed a better functional outcome (better final visual acuity) and we also demonstrated an improvement in the anatomical status (OCT thinner macular area) after PPV with ILM peeling. The cases with worst final visual acuity were related to the impairment in diabetic macular ischemia.

B-1. OCT and Diabetic Macular Edema Surgery

In a series of patients with DME submitted to surgical treatment and OCT control, Massin and associates (2003) concluded that vitrectomy was beneficial in eyes with diffuse DME combined with vitreomacular traction but not in eyes without traction. OCT allowed diagnosis of subtle vitreomacular traction and provided precise preoperative and postoperative assessment of macular traction. The OCT findings and the postoperative evolution confirmed the hypothesis of Lewis and colleagues

(1992), who suggested that the taut thickened posterior hyaloid exerted tangential vitreomacular traction that induced or exacerbated DME. The thickening of posterior hyaloid may have been partly due to the structural changes in the vitreous cortex reported in diabetic patients (Sebag et al, 1992; Stitt et al, 1998) and to infiltration of the hyaloid by cells of glial and epithelial origin (Jumper et al, 2000).

In our point of view, OCT proved to be a useful tool that provided precise objective assessment of macular thickness before and after vitrectomy for diffuse DME. Although this rare condition can be suspected on biomicroscopy in most cases, OCT also confirms the diagnosis by providing an objective image of vitreomacular traction; in addition, OCT may help to detect more subtle traction not visible on biomicroscopy.

In the current study, 94 % of eyes had decreased DME according to OCT foveal thickness analysis. The final postoperative mean foveal thickness determined by OCT was significantly thinner than the preoperative foveal thickness, and the postoperative foveal thickness was significantly and progressively thinner than the preoperative foveal thickness. The results of this study have demonstrated that vitrectomy with ILM peeling, is effective in reducing DME. OCT analysis showed that macular thickness measurements were repeatable within a session and over different independent sessions. OCT had a key role in monitoring the foveal thickness evolution during the follow-up period.

C. Histopathology and Clinic-Pathological Correlation

Smiddy and associates (1989) removed posterior cortical vitreous in patients submitted to vitrectomy for an impending MH (Stage 1). No specific attempt was made to remove the ILM at the time of surgery. All specimens showed vitreous condensates and only few pieces had fragments of ILM. By TEM, segments of ILM were associated with fibrous astrocytes.

Hee-Seong Yoon and associates (1996) observed a proliferation of cells on the inner surface of the ILM and the most frequent cell types observed by TEM were myofibroblasts and fibrocytes followed by RPE cells and fibrous astrocytes. RPE cells and fibrous astrocytes appeared to have undergone myofibroblastic differentiation. ILM was present in most pieces. Yoon concluded that their findings supported the hypothesis that tangential traction is produced by "the a-cellular prefoveal vitreous and possible contraction of the cellular constituents in the prefoveal vitreous". However, there is no direct evidence to support this interpretation (Ferris, 1997). What this study does, in fact, demonstrated is that in stage 2, 3 and 4 macular holes, the associated "epiretinal membranes" are in fact the ILM, and a more logical conclusion would be that changes in the ILM are responsible for tangential traction, not changes in the prefoveal vitreous. If the original conclusions of Yoon and associates (1996) and of Gass (1995) were correct (Ferris, 1997), there would be no need to remove the ILM at the time of surgery, and removal of the prefoveal vitreous alone would be sufficient to relieve tangential traction. In fact, it is now evident that the ILM as well as the prefoveal vitreous should be removed if the traction on the neurosensory retina is to be relieved.

Histopathological studies (Smiddy et al, 1989; Hee-Seong Yoon et al, 1996; Eckardt et al, 1997; Messmer et al, 1998; Sadda et al, 1999) showed that collagen fibers associated with epiretinal membranes and scanty amount of several cells types such as myofibroblasts, RPE cells, fibrous astrocytes, macrophage or fibrocyte-like cells were the principal findings of collected tissue, during MH surgery. They concluded that, glial cells and newly formed collagen may play an important role in MH formation by exerting tangential traction regardless of the underlying disease process and glial cells may also be involved in healing of the retinal defect.

Our histopathological study corroborates the findings encountered previously. Nevertheless, by TEM analysis we also found that some fragments of neurosensory retina elements could be associated with ILM on its retinal face.

Kwok and colleagues (2001) studied ILM removed with ICG staining in MH surgery. A single layer of myofibrocytes-like cells associated to ILM was observed and basement membrane component was intensely stained by periodic acid-Schiff. The end feet of Müller cell fibbers were showed associated with ILM by glial fibrillar acidic protein immunocytochemical staining. Type I and type IV collagen was also positive in most cases. Haritoglou and associates (2001) contested that cellular elements such as plasma membranes of Müller cells and other glial cells are common features of the ILM.

Ezra and associates (2001), characterized immunocytochemically the neural and glial components of idiopathic full-thickness MH opercula. In all, GFAP, vimentin and cellular retinaldehyde binding protein-positive glia were present but were negative for rod photoreceptors. These findings provide further evidence that a significant proportion of macular holes arise from avulsion of foveal neural tissue ("true

opercula") rather than from a pure foveal dehiscence without tissue loss or avulsion of only superficial inner-retinal glial tissue ("pseudo-opercula").

It should be kept in mind, however, that ILM is comprised primarily of the basement membrane derived from Müller cell footplates, and it is not surprising that ILM peeling might disrupt some of these footplates. ILM peeling often results in petechial haemorrhage around the fovea, but ILM peeling also produced mild pale retinal edema in the entire area where the ILM was removed. Smiddy et al (2001) suggest that shearing injury to Müller cell footplates that adhere to the ILM during ILM removal might produce neurosensory retinal damage. It is important to note, that in our study we also found neurosensory retinal elements on the retinal face of few ILM with a worst final best-corrected visual acuity of those eyes at the end of follow-up. This hypothesis is supported by observations that focal macular electroretinograms are transiently depressed in eyes with ILM peeling compared with no dissection, with limited b-wave recovery implicating possible Müller cell injury, because Müller cells contribute to generation of the b wave (Terasaki et al, 2001).

In the present study, stereological analysis performed on ILM obtained during surgery showed that the mean of ILM thickness in MH group was greater than those observed in DME group and in normal retina, with significant difference. Perhaps that fact was due to a structural separation of the ILM histological components or to a break in the normal metabolism of that complex basal membrane.

In the literature, there were few studies about the ultra-structural aspects of the ILM in DME. Gandorfer and associates (2000) observed by TEM that the cellular elements attached to the ILM could be identified as fibrous astrocytes and collagen fibbers of various diameters, suggesting the presence of native vitreous collagen and newly formed collagen. Macrophage-like cells, myofibroblasts, fibrocyte-like cells and retinal elements were not found. Other study by immunocytochemistry characterization of posterior hyaloid removed during diffuse DME, showed type II collagen (Jumper et al, 2000).

In three patients of the current study, the histological analysis of the ILM, showed the presence of neurosensory retinal elements in the samples examined by TEM. Those elements, was on the retinal side of the ILM. Often they were accomplished with other cellular elements such as glial cells, macrophages, and epithelioid-like cells, on the vitreous face of the internal limiting membranes. Only one patient had a better best-corrected visual acuity, and the others presented a worst visual acuity at the end of the follow-up. It may even be speculated that a cellular injury to the adjacent retina should be responsible to induce visual deficits rather than improvement of visual function or that the CDME is responsible for the migration and location of those cells, adjacent to the peeled area.

We measured the ILM in DME and we found differences in thickness between normal and diabetic ILM specimens. Results showed that the mean of ILM thickness in DME group was greater than those observed in normal retinas. The metabolic deviation of the diabetes, which stresses the retina during the progressing of diabetic retinopathy (DR), may explain the structural thickness of the ILM in the DME. If diabetes is responsible for the structural alterations of the basal membranes of micro-vessels during the course of DR, with capillary closure in the arterial side of the capillary net and microaneurysm formation in the venous side (Cunha-Vaz, 1972), perhaps the same phenomena explain the thicken aspect of basal membrane of the Müller cells in our study.

VII. CONCLUSIONS

MH group:

1. PPV with ICG-assisted ILM peeling is a useful technical procedure in the surgical treatment of MH. The rate of hole closure was 90 % and the surgical option of this one year prospective study, showed a better mean final best-corrected visual acuity, in eyes with closed holes.

2. OCT was diagnostic and used as a non-invasive alternative to evaluate the foveal status in the eyes with MH, before and after surgical treatment. The anatomic closure evolution of macular holes was very well analysed and documented with OCT in the postoperative time.

DME group:

1. This prospective clinical one-year follow-up study, showed the benefits of the ILM extraction during PPV for surgical treatment of CDME. The extraction of the ILM contributed to the thicken diminution and clinical resolution of the DME as the achievement of the clinical success rate (better visual acuity).

2. OCT examination allows more accurate qualitative follow-up of the changes in retinal profile, and an objective quantitative assessment of small changes in macular thickness: significant thinner macula in the OCT at the 1st month after surgery ($p = 0.00016$) compared with the significant better mean best-corrected visual acuity only at the 6th month of follow-up ($p = 0.05$).

3. Histological pieces analysed by LM and TEM, showed that not all specimens had fragments of ILM. Sometimes specimens were thicken hyaloid or epiretinal membranes.

Both groups:

1. The clinic-pathological correlation indicated that the presence of neurosensory retinal elements on ILM, showed a tendency to a worst visual acuity. In both group, retinal elements were seen on the retinal side of some ILM and those eyes presented a worst final best-corrected visual acuity.

2. The ILM thickness in MH group was greater than the ILM in DME group ($p = 0.03$), which was thicken compared to the ILM in the normal retinas ($p = 0.00003$).

VIII. CONCLUSIONES

Grupo de Agujeros Maculares:

1. La vitrectomía posterior por vía pars plana asociada a la tinción de la membrana limitante interna con verde de indocianina es un procedimiento de utilidad para el tratamiento quirúrgico de los agujeros maculares. En el presente estudio prospectivo de un año, la tasa de cierre anatómico fue de un 90% y la mejor media de la agudeza visual final corregida se obtuvo en los ojos con éxito anatómico.

2. La tomografía de coherencia óptica contribuyó para el diagnóstico y se presentó como una alternativa no invasiva para la evaluación del estado foveal en el pre y postoperatorio. Asimismo, permitió documentar y analizar la evolución del cierre anatómico de los agujeros maculares durante el seguimiento post quirúrgico.

Grupo de Edema Macular Diabético:

1. El presente estudio clínico prospectivo de un año demostró los beneficios de la extracción de la membrana limitante interna, mediante vitrectomía posterior por vía pars plana, como tratamiento quirúrgico del edema macular crónico diabético. La extracción de la membrana limitante interna conllevó a una disminución del engrosamiento de la retina y la resolución clínica del edema macular, así como también a una mejor agudeza visual.

2. La tomografía de coherencia óptica permitió un detallado seguimiento cualitativo del perfil de la retina y un análisis cuantitativo objetivo de las pequeñas variaciones del espesor macular. El espesor de la macula ha disminuido significativamente al final del primer mes del postoperatorio ($p = 0,00016$) comparado con una mejoría significativa de la agudeza visual corregida a partir del sexto mes de seguimiento clínico ($p = 0,05$).

3. Mediante microscopia óptica y microscopia electrónica de transmisión, el estudio histológico mostró la ausencia de fragmentos de membrana limitante interna en algunos de los especimenes analizados. Algunas piezas histológicas eran membranas epirretinianas o la hialoides posterior engrosada.

Ambos Grupos:

1. La correlación clínico-patológica indicó que la presencia de elementos de la retina neurosensorial en la membrana limitante interna resulta en una peor agudeza visual. En ambos grupos, elementos de la retina neurosensorial fueron observados en la cara retiniana de algunos especimenes de membrana limitante interna que fueron extraídos de ojos que presentarían una peor agudeza visual final corregida.

2. El espesor de la membrana limitante interna observado en el grupo de agujeros maculares fue mayor que el observado en el grupo de edema macular diabético ($p = 0,03$) que, a su vez, fue mayor que el espesor observado en la membrana limitante interna de la retina normal ($p = 0,00003$).

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