Diagnostic and surgical techniques

Controversies over the role of internal limiting membrane peeling during vitrectomy in macular hole surgery

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Abstract
Surgical management of an idiopathic macular hole consists of vitrectomy to release vitreofoveal traction and intraocular tamponade to flatten and reappose the hole’s edges. The intentional atraumatic removal of the internal limiting membrane has been proposed as cost-effective option in macular hole surgery. The internal limiting membrane contributes to tangential traction at the edges of the hole and acts as a platform on which glial cells proliferate. Removal of the internal limiting membrane increases the elasticity of the denuded macula and improves the anatomical success rate; however, the visual consequences of this surgical maneuver are still not fully known. We discuss the beneficial and adverse effects associated with internal limiting membrane peeling in macular hole surgery, highlighting the internal limiting membrane’s role in macular hole etiology and pathogenesis and the anatomical and functional findings after its removal.

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1. Introduction
A macular hole (MH) is a vitreomacular interface disorder with a complex mechanical pathogenesis. The formation of a macular hole depends on the peculiar morphology of abnormal anteroposterior and tangential vitreous traction from an incomplete posterior vitreous detachment.44

The fovea mainly consists of photoreceptors and their axons covered by a cap of Müller cells. The internal limiting membrane (ILM) is rather thick at the macula but becomes thin over...
the fovea. Generally, at sites where the internal limiting membrane is thin, the vitreous fibers are more anchored to the retinal tissue and may exert greater tensile forces.

An MH may form when posterior vitreous detachment (PVD) occurs, but the vitreous still tenaciously adheres to the edges of the fovea.\(^{34,87}\) This phenomenon is possible when the liquefaction of the vitreous is not accompanied by a simultaneous weakening of the adherence of the posterior hyaloid to the ILM at the fovea and optic nerve.\(^86\)

Optical coherence tomography (OCT) reveals, before the formation of MH, the presence of a partially detached ring of the vitreous extending from the fovea, where it remains attached to the papilla and to the vascular arcades on one side, and to the fovea in the center. A pocket of vitreous fluid is also visible immediately in front of the macula.\(^{34,87}\) Thus, passive movements of the vitreous fluid in the precortical vitreous pocket, along with the contraction of the posterior hyaloid adhering to the fovea, create anteroposterior traction.

The ILM, the layer that defines the transition between the retina and the vitreous body, is composed of the internal expansions of Müller cells and by a basement membrane made primarily of collagen fibers, glycosaminoglycans, laminin, and fibronectin connected to peripheral fibers of the cortical vitreous. Under certain conditions, the focal traction of the vitreous adherent to the central ILM can break the retina at its thinnest point.\(^{78}\) Moreover, the ILM can act as a scaffold for cellular proliferation. Stiffening, distortion, and enlargement of the MH rim are consequences of epimacular glial and Müller cell proliferation through the retinal hole and over the ILM surface. After the formation of an MH, enlargement is largely caused by tensile shear strain from shortening of the ILM edges causing tangential traction. Moreover, stiffening and thickening of the ILM are important contributory causes of other pathologic macular conditions such as epiretinal membranes, diabetic tractional maculopathy, vitreomacular traction (VMT), and myopic traction maculopathy.\(^{30}\) The introduction of the intentional removal of the macular ILM resulted in a meaningful improvement in the anatomical success rate in the surgical treatment of an MH and a cost-effective option for the treatment of this disorder.\(^{56,88}\)

In the last 10 years, ILM peeling during surgery for an MH has become a routine step and is performed by most surgeons. With the advent of modern spectral-domain (SD) OCT, however, one can now see abnormal structural changes to the inner retinal surface with ILM peeling, suggesting possible progressive retinal damage. Moreover, some clinical studies found adverse functional events that have given rise to concerns regarding the safety of ILM peeling. In this review, we describe the current understanding regarding the pathogenesis of MHS and their treatment, paying particular attention to functional and anatomical outcomes related to ILM peeling.

2. Discussion

2.1. Current classifications of vitreoretinal interface diseases

Gass classified the 4 stages of MH evolution (Fig. 1)\(^{32,33}\) Stage 1 corresponds to a central yellow spot visible with the ophthalmoscope that is associated with a loss of the foveal depression (stage 1a). This is followed by the formation of a yellowish reflection with a ring shape (stage 1b). Stage 2 is characterized by the formation of a small hole (<400 μm), often accompanied by the formation of a visible operculum, which is the roof of the cysts seen in earlier stages. Stage 3 is characterized by the widening of the hole to more than 400 μm in diameter. In this phase posterior vitreous detachment has not yet occurred, and it is still adhered to the papilla and around the MH. Stage 4 is the same as stage 3 after a complete PVD.

With the introduction of SD-OCT, it became possible to observe the relationship between the vitreous and the macula. Abnormal adhesion between the vitreous cortex and fovea may deform the retinal surface in different ways depending on the shape and extension of the adhesion. Stage 1 of an MH corresponds to aspects of vitreofoveal adhesion as part of vitreous macular traction syndrome.

SD-OCT reveals that anteroposterior traction after an incomplete pathological PVD causes the formation of an MH in the early stage. Tangential traction becomes important in
the later stages and causes enlargement of the hole. Stiffening and shrinking of the ILM seems to be the main causes of MH enlargement.

The international study group directed by Duker and colleagues proposed a new anatomic classification of vitreoretinal interface anomalies based on the use of SD-OCT.15 This classification defines 3 main conditions: vitreomacular adhesion, VMT, and an MH. Vitreomacular adhesion is not a pathological condition because the fovea is not deformed by vitreous traction, and vision is usually not disturbed. Scientists believe that this aspect is the first stage of PVD and occurs commonly after the age of 40 years.22 In VMT, the vitreal adhesion causes distortion of the foveal contour with visual impairment. This can be subclassified according to the size of the vitreal adhesion as focal VMT if the dimension of the adhesion is less than 1,500 μm and as large VMT if the adhesion is greater than 1,500 μm and can be classified as isolated or concomitant. A full-thickness MH is characterized by a macular lesion with interruption of all retinal layers extending from the ILM to the retinal pigment epithelium (RPE). It is subclassified according to the size of the hole determined by OCT and by the presence or absence of VMT. In the first case, an MH can be classified as small (≤250 μm), medium (250–400 μm), or large (>400 μm); according to the state of the vitreous. A MH is classified as with or without VMT. It can also be classified as a primary or secondary full-thickness MH.

The term “idiopathic MH” has been abandoned, as it is now well known that an MH is caused by vitreous traction.

2.2. Müller cell metabolism and the pathophysiology of the internal limiting membrane

The term “inner limiting membrane” of the retina was coined several years ago to define the innermost layer of the retina composed of internal expansions of Müller cell footplates and a cuticular fibrous layer. An ultrastructural analysis of the human retina shows that the ILM appears as a homogeneous, periodic acid-Schiff–positive basement membrane; its retinal surface is markedly irregular, whereas its vitreal surface is smooth.25

The outer portion of the cuticular layer is formed by a dense fibrillar meshwork that forms the basal membrane of the Müller cells; the inner portion is composed of a loose net of fibrils made of mucopolysaccharides and proteins joined by vitreal fibrils. Its composition consists of type IV collagen, laminin, fibronectin, glycoproteins, and glycosaminoglycans.25

The ILM has a thickness of 400 nm at the peripheral retina, but the thickness increases to about 1,400 nm in the macular area. The cuticular layer of the ILM thins over the center of the fovea, optic nerve, and major vascular arcades. At these sites, the vitreous fibers are directly and more tenaciously anchored to the retinal tissue and may exert a greater tensile force. Müller cells (also called radial glial cells) are the most abundant glia in the retina and have an active role in retinal function. They possess a cell body located in the inner nuclear layer and a multitude of cellular processes that span the entire thickness of the neurosensory retina. They form the inner retinal surface at the level of the ILM and ensheath every type of cell body and process of the retinal neurons.

At the level of the photoreceptor layer, Müller cells enlarge and form the external limiting membrane. At the center of the fovea, a thickening of the Müller stratum with a cone-like appearance connects the ILM with the external limiting membrane. This is the so-called Müller cap that is also a deposit of xanthophyllin, which gives the operculum its characteristic yellow color in the early stages of an MH.31

Besides mechanical protection, there are a multitude of functional interactions between Müller cells and neurons such as extracellular ion homeostasis, glutamate recycling, and the exchange of waste products with the underlying ganglion cells. Müller cells possess numerous voltage-gated channels and neurotransmitter receptors such as sodium-potassium channels, gamma-aminobutyric acid receptors, and glutamate receptors, especially in their end-feet neurons. They are therefore able to modulate depolarization of the underlying neuronal cells. Their ability to modulate neuronal depolarization is also expressed through their ability to modulate the concentrations of potassium (K⁺), hydrogen (H⁺), bicarbonate (HCO₃⁻), and sodium (Na⁺) ions. Among the voltage-gated channels, the primary channel is the K⁺ channel, which regulates membrane conductance; with respect to H⁺ ions and the acid-base balance, there is an electrogenic Na⁺/HCO₃⁻cotransporter and an Na⁺/H⁺ exchanger.63,64

Müller cells also act to provide metabolic support and protection for the neurosensory retina. They mediate nerve cell protection by releasing basic fibroblast growth factor, by the uptake and degradation of glutamate and by secreting the antioxidant glutathione.53 Animal studies have shown that these glial cells are partly able to regulate retinal blood flow in response to neuronal needs. This is performed through the local release of nitric oxide and K⁺ and is facilitated by the fact that the terminal processes of Müller cells often surround and wrap around retinal blood vessels.66

Müller cell proliferation is an inflammatory reaction that heals the retina after a mechanical or inflammatory insult and to protect the neuroretinal layers from mechanical stimuli. For example, it offers protection against photoreceptor apoptosis induced by passive movements of the retina in the case of vitreal traction at the posterior pole. Neurovascularization is also mediated by Müller cells during hypoxic conditions via the release of vascular endothelial growth factor and transforming growth factor beta, or via direct contact with endothelial cells.67

Müller cell reactivity may have negative effects on vision. Changes that occur in Müller cells after the surgical procedure of ILM peeling could produce the phenomena of intraretinal fibrosis, which could in turn cause alterations in neuronal intraretinal connections and in the metabolism of macular photoreceptors. Moreover, this phenomenon may be complicated by epiretinal fibrosis because the ILM provides a scaffold for the adhesion and following pathological proliferation of glial cells.35,41,62 Müller cells, and specifically their relation with the ILM, appear to be key elements in the pathogenesis of all tractional maculopathies and in the development of an MH.

2.3. Theories on the etiology of macular holes

The most widely accepted hypothesis on the genesis of idiopathic MHs is that initial direct anteroposterior traction is exerted by the posterior vitreous cortex on the foveal area.44 After the formation of a hole, tangential traction from
tightening of the ILM becomes predominant in causing its enlargement. In the early stages of MH formation, incomplete perifoveal vitreous detachment is evident around the posterior pole. Anteroposterior traction occurs from dynamic traction created by an anomalous vitreofoveal adhesion that coexists with premacular vitreous separation and is often preceded by a precortical liquefied vitreous pocket. \(^{34,87}\)

The vitreous fluid contained in the precortical vitreous pocket slowly passes through the optic disc, causing the separation of the cortical vitreous from the posterior pole. In eyes predisposed to MH formation, however, the cortical vitreous remains firmly attached to the fovea until the avulsion of the Müller cap. OCT images show that the posterior hyaloid draws a convex shape boundary where it is attached to the fovea. The contraction of collagen fibers oriented longitudinally causes progressive anteroposterior traction.\(^{34,87}\)

A recent study on the early stages of impending MHs showed that anteroposterior traction is stronger if the area of perifoveal vitreous detachment is small. \(^{78}\) Tangential traction becomes important only after MH formation. Tangential traction results from contraction of the residual vitreous that remains over the fovea after PVD and follows the proliferation and invasion of Müller cells over the ILM.\(^{33}\)

This hypothesis is confirmed by the fact that an MH tends to enlarge, although the anteroposterior vitreofoveal attachment is released, and the operculum is completely separated from the retina. On the other hand, the spontaneous closure of a complete full-thickness MH is an uncommon event.\(^{23}\)

As mentioned before, the central and inner layers of the foveola are supported by a thickening of the Müller stratum, which has a cone-like appearance (Fig. 2). This cone-shaped cap serves to keep the photoreceptors together. It stretches nearly completely across the thickness of the retina and probably contributes to anchoring the photoreceptors to the bottom of the fovea. The Müller cap may have a role in the genesis of an MH because it is tightly adherent to the posterior vitreous cortex and transmits tensile force to the external limiting membrane.\(^{31}\)

A split in the Müller cap begins with a break in the external limiting membrane over the underlying foveolar cones. This may be seen on OCT in the configurations referred to as foveoschisis and a foveal cyst that precede the formation of an MH (a stage 1 MH). When traction creates a dehiscence in the roof or walls of the cyst, an MH is formed. The dehiscence is asymmetrical at first because the vitreous remains attached to one side of the MH and to the Müller cone. Then, the traction causes an avulsion of the Müller cap from the retina creating the operculum, which remains suspended on the posterior vitreous cortex.

An ultrastructural examination of the operculum has shown fragments of glial and Müller cells, the vitreous cortex, and in 40% of cases, photoreceptor debris.\(^{74}\) This finding demonstrates that a certain amount of photoreceptors may be loosened after MH formation; however, most photoreceptors remain functional at the bottom of the foveola but shrink centrifugally and thereby form an MH.

In an ultrastructural study of 100 ILMs that were peeled after MH surgery, 57% of glial cells, Müller cells and fibroblast proliferation producing new collagen fibers were found on the vitreal side of the ILM.\(^{72}\) On the ILM of a stage 4 MH, the amount of collagen fibers produced was greater than the amounts produced in stage 2 and 3 MHs. This suggests that a certain amount of the vitreous remains over the inner retina, even if a spontaneous PVD occurs, and that the amount of vitreous on the ILM is proportional to the stage of the disease.

After the formation of an MH, a local inflammatory reaction may activate cells, recently identified as trans-Müller cells, that differentiate into fibroblasts and myofibroblasts, proliferate along the surface of the ILM, and deposit new collagen types I, III, and V. In these patients, myofibroblasts and fibroblasts produce an epiretinal membrane that covers Fig. 2 – (A) The central and inner layers of the foveola are supported by a thickening of Müller stratum that has a cone-like appearance: the Müller cap. It may have a role in the genesis of a macular hole because it is tightly adherent to the posterior vitreous cortex and transmit the tensile force to the ELM. (B,C) The split of the Müller cap begins with a break of the ELM with the formation of a “foveal cyst” that precedes the formation of a macular hole. (D) The vitreal traction cause an avulsion of the Müller cap from the retina creating the operculum which remains suspended on the posterior vitreous cortex.
the surface of the ILM and contributes to enlargement of the hole, even when vitreous traction is released.8

Another study performed by combining OCT and scanning laser ophthalmoscopy has highlighted the role of vitreoschisis, documented in 53% of MH cases.73 The perifoveal vitreous cortex splits into 2 portions; the first portion is posterior and adheres to the retina, and the second portion is in the context of the detached vitreous. In MHs vitreoschisis occurs in the vitreous cortex behind the level of the hyalocytes. This explains why there is no clinically evident epiretinal membrane in these patients.

Thus, a large amount of evidence supports the concept that the particular anatomical configuration of the fovea predisposes this thinner retinal area to traction forces exerted in longitudinal and tangential directions if an anomalous limited posterior vitreous detachment occurs.8,72,73

VMT can resolve spontaneously over time without intervention in a significant number of eyes. The probability of relaxation is greater for diameters of adherence less than 400 μm, low reflectivity of the posterior vitreous, foveal thicknesses less than 500 μm, and wide angles of insertion between the vitreous and the retina.89 Observation is recommended for at least 6 months before initiating any treatment. Some studies have reported that, after 6 months of observation, the incidence of VMT resolution is approximately 9%–11%.12,39,92 however, others reported that if observation exceeds 12 months, the incidence of resolution may reach 50%.13 Spontaneous resolution of an MH is unlikely. A follow-up that lasted 6 years found resolution of small MHs in 9% of cases.12

Visual acuity determined after the formation of an MH tends to worsen over time. One year after the formation of a hole, 2 Snellen lines are lost in 30% of stage 1 holes, 68% of stage 2 holes, 29% of stage 3 holes, and 13% of stage 4 holes.12

In 1991, Kelly and Wendel suggested a strategy for treating MHs based on a 3-port pars plana vitrectomy with removal of the posterior hyaloid and reapposition of the MH edges by flattening them with intraocular tamponade. The goal of the surgery is to relieve all anteroposterior traction in the macular region. Two large randomized controlled trials (RCTs) demonstrated a clear benefit from this type of surgical management for stages 2 to 4 MHs according to the rate of anatomical closure, final near acuity results, and Snellen acuity results: the Vitrectomy for Macular Hole Study27,48 and the Moorfields Macular Hole Study.23

Macular hole closure was achieved in 69% of eyes in the Vitrectomy for Macular Hole Study and in 77% of eyes in the Moorfields Macular Hole Study that were randomized to surgery, versus only 4% and 6% of eyes, respectively, randomized to observation alone. There was no attempt to peel the ILM in these studies. The use of small-caliber vitrectomy has become recommended for at least 6 months before initiating any treatment. There was no attempt to peel the ILM in these studies. The use of small-caliber vitrectomy has become recommended for at least 6 months before initiating any treatment.

In a large retrospective comparative clinical study of 160 eyes with MH of less than 6 months’ duration showed that anatomical closure was achieved in 100% of patients in 116 eyes treated with ILM peeling, whereas anatomical closure of holes was obtained in 82% of patients in 44 eyes not treated with ILM peeling. In the latter group, reopening of the hole occurred in 25% of the patients; however, in the first group, this complication never occurred.

Furthermore, a third group of patients with MHs of more than 6 months was treated with ILM peeling, and 63 of 65 holes (97%) were closed, with an improvement in vision of 2 or more Snellen lines in 65% of eyes.7 In a comparative clinical study of 39 eyes, anatomical closure of the hole was achieved in 90% of patients treated with ILM peeling, versus only 50% of patients treated without ILM peeling. Significant visual acuity improvement occurred in 62% of patients treated with ILM peeling in comparison with 44% in the other group.26 In another study, 97 control patients who underwent pars plana vitrectomy without ILM peeling were compared with 79 patients who underwent pars plana vitrectomy and ILM peeling, with or without indocyanine green (ICG)-assisted ILM peeling. In patients that underwent ILM peeling with or without ICG, the rate of closure was 97%. In the control group, the rate of closure was 77.3%. The use of ILM peeling was associated with a significant improvement in vision in 77.3% of patients; however, vision improved in only 65% of the controls.26 Other studies, however, have found no significant difference in the rate of closure of MH in patients treated with or without ILM peeling.57

The rationale for peeling the ILM is to eliminate any tangential traction component implicated in the genesis of MH formation and to stimulate macular wound healing. Müller cells and fibroblasts proliferating before or after MH formation can cause stiffening of the retina around the MH, thereby hindering closure.7,19,54 ILM peeling may also reduce the possibility of late reopening of a surgically closed hole by removing any scaffold for myofibroblast reproliferation. A large study of 877 eyes reported that the late reopening rate was 0.39% in eyes that underwent ILM peeling and 7.2% in eyes without ILM peeling.10

Furthermore, ILM peeling appears to stimulate Müller cell reactivity, which stimulates wound healing at the MH. Conflicting data exist on the predictability of the visual outcome. Concerns have been raised about the possibility of creating irreversible retinal damage by ILM peeling.

In a case series, the functional results of 29 eyes treated with ILM peeling were compared with the results obtained in 27 eyes treated without ILM peeling. Visual improvement of more than 3 lines was achieved in 79.2% of eyes with MH closure without ILM peeling, but in only 44.8% of eyes with MH closure with ILM peeling.7

A retrospective case series evaluated visual acuity in a group of consecutive patients affected by MHs before and after switching to ILM peeling. The authors noticed that eyes without ILM peeling had faster postoperative improvements in visual acuity at 3 months compared with eyes that underwent ILM peeling; however, after 6 months the visual outcomes were comparable.90

In a large case series of 193 eyes in which 23% of the eyes were peeled completely, 43% were peeled partially, and 34% were not peeled at all, the authors observed that ILM peeling is not essential for anatomic or visual success in MH surgery, but it is a means to induce gliosis that promotes MH closure. On
the other hand, excessive unsuccessful attempts at ILM peeling may decrease visual success.26

2.4. Intraoperative vital staining of the internal limiting membrane

ILM peeling has become a more widely accepted procedure since the introduction of vital dyes. Because the ILM is poorly visible, its identification is challenging, and its removal is difficult, even for an experienced vitreoretinal surgeon.

The complete dissection of the ILM is a technically challenging maneuver because of the difficulty in distinguishing the ILM from the nerve fiber layer with confidence. Moreover, incomplete ILM removal may cause a failure in MH closure, whereas inadvertent injury to the nerve fiber layer may cause paracentral scotomata.27

To achieve reproducible, complete, and less-traumatic ILM peeling, intraocular vital dyes have been introduced to facilitate clear identification. Available materials are usually classified as a staining material—such as ICG, trypan blue, brilliant blue G, and acid violet—or as coating material such as triamcinolone acetonide (TA) and blood.

The first among these dyes, used since 2000, is ICG at a concentration of 5 mg/mL (0.5%), which provides stark contrast between the stained ILM and the unstained retina.9,16,45 The use of this vital dye represented an important surgical development because it improved the rate of primary hole closure from 73.5% without ICG staining to 91.2% with ICG staining.55

Some clinical studies reported that ICG-assisted ILM peeling did not compromise the visual result and did not have a negative effect on retinal function.15,82,94 On the other hand, other clinical studies found that ICG-assisted surgery might have adverse effects on functional outcomes.4,22,36,40,91

Usually these studies reported that a lower number of patients had visual acuity improvement after surgery when ICG staining was performed. Moreover, postoperative development of peripheral visual field defects may develop.5 These support the hypothesis that ICG could be toxic to the retina if injected directly into the eye and could compromise the functional success of the treatment.30

A direct toxic effect has been highlighted by numerous animal and in vitro studies on many cell populations, including RPE, retinal glial, and ganglion cells.29,93 Toxicity to the RPE makes the use of ICG dangerous when it is injected directly above the MH because of the risk of it spreading beneath the retina.

ICG also seemed to have an indirect toxic effect on the retina because of the hypo-osmolarity of the injected solution and a phototoxic effect triggered by the endoilluminator.22,69 Moreover, other studies have established that ICG can persist for many months after surgery, so the phototoxic effect could persist.47

A morphological examination of ILM samples after the use of ICG demonstrated that the cleavage plane of the membrane is deepened, and its removal also removes layers of ganglion or Müller cells.95 Far more cellular debris was seen on the retinal sides of ICG-stained ILM than on unstained specimens.

Thus, the use of ICG was thought to explain the reduction in ganglion cell thickness, especially in the macular temporal region, in the postoperative period after vitrectomy with ILM peeling. Later, it was found that this phenomenon, as well as the phenomenon of visibility of a dissociated optic nerve fiber layer (DONFL), occurs regardless of the dye used.93,60 Indeed, Christensen and colleagues found that the percentage of cases with a DONFL appearance was the same in ICG-peeled and trypan blue-peeled eyes, which suggests that the ILM peeling procedure is causal rather than the dye used.93

Surgeons using ICG should select a concentration below 0.5 mg/mL, preferably below 0.05 mg/mL. The exposure time should be the minimum possible, and the endoilluminator should be kept as far from the fovea as possible. Hypo-osmolar solutions should be avoided, with 5% glucose used as a diluent rather than distilled water.

After initial enthusiasm, most clinicians discontinued the use of ICG as an intraoperative vital stain because of concerns about toxicity. Other vital dyes were later introduced to replace ICG: trypan blue 0.15%, brilliant blue G, TA, and recently, acid violet 17 (C6H3(N4Na2O8S2).75

Trypan blue is not specific for the ILM, but sufficiently stains the inner retinal surface and provides useful contrast between the colored surface and the underlying unstained layers. It appears to be less toxic than ICG, as shown by studies that highlighted the best functional results and lower incidence of central scotoma in groups of patients with idiopathic MHs that were treated by vitrectomy with ILM peeling and stained with trypan blue rather than ICG.59

Brilliant blue G, another vital dye that was introduced after trypan blue, has a good safety profile, provides significant anatomical and functional postoperative results,68 and has the peculiar characteristic of staining the ILM and not the rest of the retina as satisfactorily as ICG and not the rest of the retina.

TA, a synthetic glucocorticoid that can be used inside the eye, has the consistency of a whitish powder that forms a deposit on the retina. TA can be used in a nonspecific way to distinguish epiretinal membranes and the posterior hyaloid from the retina, and the ILM from the underlying retinal layers, but has the major drawback of dirtying the tips of instruments and is an absolutely noneselective dye.

TA is considered safe,73 except for possible transient ocular hypertension; however, studies exist that highlight long-term toxicity when used at high concentrations.71 In animal species, some toxic effects have been shown in RPE cells, retinal Müller glial cells, and retinal neurosensory cells.97

Finally, recently another vital dye has been introduced: acid violet 17, which is specific to the ILM and allows its clear intraoperative visualization. Acid violet 17 seems to be easier to use and seems to offer greater contrast than brilliant blue G. Acid violet 17 was shown to be safe for the retina at concentrations of 0.25 and 0.50 g/ L after intravitreal injection; however, further studies are required to confirm its long-term safety.11

3. Results

3.1. Concerns about the anatomical and functional effects of internal limiting membrane peeling

The first sources of concern were anatomic changes of the macula that occur after peeling and during the first months of
follow-up. Soon after peeling, the macula assumes a whitish color, frequently with small hemorrhages in the area of the denuded macula. This appearance is probably from swelling resulting from interruption in the axonal transport of ganglion fibers that run under the ILM. Swelling of the arcuate retinal nerve fiber layer is followed by a DONFL appearance, which is sometimes visible (in 43% of patients who underwent surgery with ILM peeling) on fundus examination with blue light a few weeks or months after surgery.

On OCT, a notch or dimples in the inner retinal layers may be detectable. These dimples probably form in areas where there are Müller cell attachment plaques that are thicker and more adherent to the ILM. In enface OCT frames, the dimples appear as concentric dark spots in the area of the macula denuded from the ILM.

After ILM peeling and MH closure, the distance between the fovea and the optic disk is shortened and the foveal contour appears asymmetrical. The displacement of the fovea toward the disk thickens the retina on the nasal side and thins the retina on the temporal side. Thinning on the temporal side is increasingly evident months after surgery (Fig. 3).

Retinal nerve fiber layer thinning may result from an injury with the subsequent degeneration and apoptosis of Müller or ganglion cells. Despite these anatomical changes, the effects on vision are uncertain. Some have documented the occurrence of paracentral microscotomata measured with microperimetry above retinas denuded from the ILM. These scotomata may be deep or multiple, may coexist with good visual acuity, and are usually asymptomatic; however, they sometimes may worsen the quality of vision, such as reductions in reading speed or contrast sensitivity. The cause of these microscotomata may be direct trauma to retinal cells induced by forceps and the mechanical stretching of the ILM, or they may be caused by secondary degenerative phenomena. Internal limiting membrane peeling could induce the degeneration of some arcuate fibers directed toward the optic nerve or apoptosis of some Müller cells that lie beneath the area of the peeled retina. Haritoglou and coworkers demonstrated that fragments of Müller cell end feet might remain attached to the ILM after peeling. This maneuver probably damages a certain percentage of these cells.

Damage to the retinal nerve fiber layer may be more noticeable with the use of dyes such as ICG. Moreover, different ILM peeling techniques performed during surgery for idiopathic MHs may influence the rate of the postoperative extent of a DONFL appearance. The use of a forceps pinch-peel technique to peel the ILM was found to be less traumatic to the nerve fiber layer than the use a diamond-dusted membrane scraper.

Tadayoni and coworkers used an OCT/scanning laser ophthalmoscopy microperimetry device and a customized pattern of points to test the light sensitivity of the central area of the macular region in patients before and after surgery for MH. They demonstrated a decrease of 3.4 dB in mean differential light threshold sensitivity at the fovea of patients who underwent ILM peeling compared with patients who did not. This finding indicates that, after ILM peeling, the retina needs more than twice as much light to see a microperimetric spot as before peeling. Furthermore, in most patients who underwent ILM peeling, Tadayoni found absolute microscotomata, which were not present in patients who did not undergo peeling.

Other researchers, however, have not confirmed changes in the differential light threshold when using a standard visual field test or microperimetry but instead have shown a significant improvement in the differential light threshold with microperimetry, as well as in reading speed and distance and near visual acuity.

Only in recent years, 3 comparative studies have been completed. They compared the anatomical and functional results obtained in groups of patients who had MH. One group was operated on in the conventional manner, and the other had ILM peeling. Kwok and colleagues published the first RCT in 2005. They compared the clinical results of a group of 26 eyes treated with ILM peeling using ICG with the results of 25 eyes treated without ILM peeling and vitrectomy alone and demonstrated a clear advantage in the ILM peeling group, with primary closure of the MH of 92.3% of eyes compared with 32% of eyes in the group without peeling. Improvement in best corrected visual acuity was 3.7 lines in the ILM-peeled group and 1.5 lines in the other group. Kwok concluded that ICG-assisted ILM peeling resulted in significantly better anatomical and visual outcomes than vitrectomy alone.

Christensen and colleagues conducted an RCT on eyes with stage 2 or 3 MHs to test the efficacy and safety of ILM peeling in
small diameter and recent MHs. They compared 25 eyes treated with conventional surgery (i.e., vitrectomy alone) with 53 eyes operated on with ILM peeling in which 18 eyes were administered trypan blue and 35 eyes were administered ICG dye. The primary closure rate was significantly higher in eyes treated with ILM peeling (100% of eyes with stage 2 MH and 89%–91% of eyes with stage 3 MH), compared with eyes operated on by vitrectomy alone (55% of eyes with stage 2 MH and 35% of eyes with stage 3 MH). The visual outcomes in eyes in which closure of the MH was obtained with a single intervention (i.e., primary closure) were not statistically significantly different between the 2 groups. Christensen concluded that ILM peeling is safe and more convenient for treating stage 2 and stage 3 MHs, with no difference between trypan blue and ICG when used to stain the ILM.

In 2011, the largest randomized, prospective, controlled trial (the Full-thickness MH and Internal Limiting Membrane Peeling Study) was published.56 Full-thickness MH and Internal Limiting Membrane Peeling Study compared a group of 66 eyes treated with ILM peeling after staining with trypan blue with a group of 65 eyes treated without ILM peeling. The trial showed that 84% of eyes that underwent ILM peeling had primary hole closure versus 48% of eyes that were treated conventionally;7 demonstrated a lower rate of reoperations with ILM peeling, and concluded that it was more cost-effective to peel the ILM because of the lower reoperation rate. There was no difference in distance acuity or near visual acuity between the groups who underwent the 2 procedures with successful hole closures.

A recent meta-analysis conducted on all main RCTs compared the anatomical and functional results in patients treated with ILM peeling. At 6 months, there was no significant difference in best corrected visual acuity; however, analysis showed a significant statistical difference in the rate of hole closure and a significant reduction in the requirement for additional surgery in favor of ILM peeling. The authors concluded that ILM peeling was highly cost-effective and may be considered the treatment of choice for patients with idiopathic stage 2, 3, and 4 full-thickness MHs.83 The RCTs have shown that the introduction of ILM peeling in clinical practice has improved the surgical results and obtains anatomical closure in more than 90% of eyes with MHs, versus 50%–70% closure in patients without ILM peeling.56,79,88

From RCTs data obtained from the literature, it appears that anatomical modifications and the suspected risk of functional damage that may occur at the maculae of patients treated with ILM peeling for MH are acceptable adverse effects after considering the benefits. In RCTs, the rates of MH in the early and late stages of development were nearly doubled when ILM peeling was performed. Furthermore, clinical experience demonstrated that it is nearly impossible to close large (greater than 500–600 μm), chronic, and myopic MHs without ILM peeling.82 Different ILM peeling procedures such as inverted flap ILM peeling (a modified procedure in which the ILM is stripped to the edge of the macula and positioned inverted or “upside-down” inside the hole) have been proposed for extremely large and myopic chronic MHs.59

Most eyes unsuccessfully treated by vitrectomy alone in RCTs were subsequently reoperated using ILM peeling and attained anatomic closure of the MH. When considering the surgical risk, possibility of postinflammatory macular edema26,70 induced by repeated surgery on the macula, and delayed anatomical closure, the practice of ILM peeling seems largely justified.

Many recent studies have shown that ILM peeling is a procedure that can cause immediate traumatic effects and progressive modifications to underlying inner retinal layers. Mechanical damage to Müller cells triggers a cascade reaction that results in postsurgical modifications in macular morphology (i.e. swelling of the arcuate retinal nerve fiber layer and DONFL appearance, and thinning and wrinkling of the temporal retina). These modifications sometimes cause functional alterations.

Most clinical studies showed that functional changes, such as a transient reduction in retinal sensitivity, appeared to be reversible and did not compromise final visual recovery. Despite the possibility that ILM peeling may cause retinal trauma and subclinical functional vision damage, the major clinical findings are in favor of its use in MH surgery.

The most recent RCTs have validated the advantages of ILM peeling in that MH closure may be obtained in 90% of patients, versus a success rate of less than 50% when it is not performed. ILM removal is associated with a higher success rate, a higher closure rate, and fewer late recurrences; however, ILM peeling offered no additional benefit when applied to eyes with an MH less than 400 μm in diameter. Closure rates of small holes (<400 μm) were equal (almost 100%) when comparing groups with and without ILM peeling in most studies.56,85 Recent evidence has demonstrated that an ILM peeling maneuver is not necessary for all types of MH.

Ocriplasmin (Jetrea; ThromboGenetics, Inc.; Iselin, NJ, USA) is a truncated form of plasmin used for its proteolytic activity against fibronectin and laminin, which cause vitreous liquefaction and consequent PVD. Recent phase 3 clinical trials have shown that a single intravitreal injection of the vitreolytic agent ocriplasmin closed about 40.1% of MHs smaller than 400 μm in diameter included in the study.81

Several studies have also shown better success rates with vitreous traction in patients with MH smaller than 250 μm. These data suggest that, for selected patients, it is not necessary to peel the ILM to achieve MH closure. On the other hand, this maneuver seems essential for large MH, even with its related adverse effects.

To improve the closure rates of MH larger than 400 μm and those in highly myopic eyes with or without retinal detachment, the “inverted flap technique” was recently introduced.59 The ILM is not completely removed. In particular, the membrane at the margins of the MH is left in place. Subsequently, the ILM remnant is inverted upside-down to cover the MH.58 The inverted ILM flap technique provides both Müller cell fragments to stimulate glial proliferation and provides a basement membrane substrate, which acts as a scaffold for cellular proliferation. Thus, these emerging concepts indicate that complete ILM peeling is unnecessary for some cases of MH.

4. Conclusions

Our review of literature shows that, although ILM peeling does not improve visual acuity in the short postsurgical period, it is
more favorable from a cost-effectiveness standpoint by increasing the likelihood of primary anatomical closure with no significant difference in functional side effects compared with no peeling. ILM peeling can cause some notable morphological changes of the macula such as thinning on the temporal side, a DOFNL, the appearance of swelling of the arcuate retinal nerve fiber layer, and a reduction in papillofoveal distance; nevertheless, no functional consequence is attributable to these anatomical modifications. Moreover, complete ILM peeling is no longer suitable for every type of MH surgery. The decision to peel or not to peel the inner limiting membrane or whether to perform complete or partial peeling depends on the anatomical characteristics of the hole and on the associated ocular conditions of the patient.

Further studies are needed on the effects of ILM peeling in eyes particularly susceptible to mechanical and metabolic damage. Eyes suffering from glaucoma, diabetes, macular degeneration, high myopia, and so on might be more susceptible to trauma from ILM peeling. These eyes could suffer additional injury from macular surgery that could impair the planned visual recovery. Eyes with a full-thickness MH are also more sensitive to any toxic effect caused by a foreign substance introduced into the eye because it allows direct contact with the RPE of the fovea. Thus, there are still disputes regarding the safety of vital dyes, and further studies are necessary to validate their use in MH surgery.

Therefore, a more accurate analysis using retinal imaging methods and functional tests might help us to understand and distinguish in which cases ILM peeling can be useful and in which cases it can be dangerous. It would be desirable to develop increasingly precise and minimally traumatic techniques to permit removal of the ILM with the least impact possible on Müller cells. Surgeons should be aware that it is necessary to limit ILM peeling in eyes with concurrent diseases and to learn to limit the extent of this maneuver, especially in retinal areas more prone to mechanical trauma such as the temporal side of the macula.

An interesting concept currently being studied is the possible role of intraoperative OCT in ILM dissection, which would suggest performing ILM peeling only when ILM pathological thickening is present, especially if associated with the presence of an epiretinal membrane.

5. Method of literature search

For this review, a search of PubMed from January 1987 through September 2015 was performed using the following terms (or combination of terms): macular hole, internal limiting membrane peeling, vitreomacular surgery, vital dye staining, Müller cells, vitreomacular diseases, and optical coherence tomography. The studies were limited to those published in English.

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