CHARLES UNIVERSITY IN PRAGUE
1ST FACULTY OF MEDICINE

SURGICAL TREATMENT OF DIABETIC MACULAR EDEMA

REPORT OF THE DOCTORAL THESIS

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Surgical Treatment of Diabetic Macular Edema

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PhD Program in Experimental Surgery
Charles University in Prague
1st Faculty of Medicine

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1. INTRODUCTION

Diabetic macular edema (DME) is the most common cause of visual impairment in diabetic patients (Klein et al. 1984; Moss et al. 1988). DME 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). The overall incidence and prevalence of DME increase with longer duration of diabetes mellitus and greater severity level of diabetic retinopathy (DR) (Klein et al. 1989). The precise pathogenic mechanisms of diffuse clinically significant DME have not been established (Bresnick 1983, Ferris and Patz 1984, Schepens et al. 1984, Nasrallah et al. 1988). The reasons discussed for the development and progress of DME are diverse. Breakdown of the blood retinal barrier and vitreomacular traction are probably the most relevant factors (Vinores et al. 1998, Sander et al. 2002). Various factors have been shown to exacerbate DME, such as fluid retention caused by cardiovascular or renal disease, uncontrolled hypertension, pregnancy, and panretinal photocoagulation (Bresnick 1986). The increasing number of individuals with diabetes worldwide suggests that DR and DME will continue to be major contributors to vision loss and associated functional impairment in the working-age population of most developed countries. The Early Treatment Diabetic Retinopathy Study (ETDRS 1985, ETDRS 1987) classified DME by its severity. It was defined as clinically significant DME (CSME) if one or more of the following features were present: (1)Retinal edema within 500 µm of the centre of the fovea, (2) Hard exudates within 500 µm of the center of the fovea, if associated with adjacent retinal thickening (which may be outside the 500 µm limit), (3) Retinal edema that is one disc area (1500 µm) or larger, any part of which is within one disc diameter of the center of the fovea. DME can be also classified as focal, diffuse, and cystoid edema, and involves three structural changes, including sponge-like retinal swelling (**Fig. 1**), cystoid macular edema (CME), and serous retinal detachment (**Fig. 2**) (Otani and Kishi 1999).

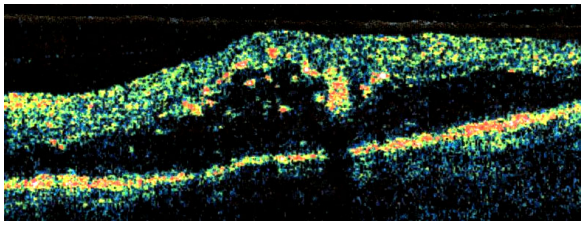


Figure 1. DME, sponge-like retinal swelling

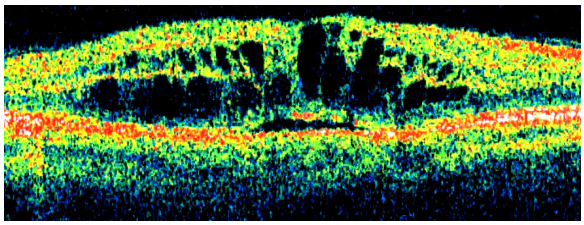


Figure 2. CME and serous retinal detachment

Although in rare cases the edema may resolve spontaneously (Yamaguchi et al. 2003), treatment is highly recommended to avoid irreparable visual loss. The ETDRS found that focal laser photocoagulation reduces the risk of the moderate visual loss for eyes with DME by 50%. Regarding the functional results, grid-pattern laser photocoagulation in diffuse DME showed limited efficacy in several studies (Bresnick 1983, McDonald et al. 1985, Olk 1986, Lee et al. 1991). The failure of laser photocoagulation in substantial subgroup of patients has prompted interest in other treatment methods, including surgical treatment with pars plana vitrectomy, removal of ILM and intravitreal application of corticosteroids. Since the pilot study of Lewis in 1992 (Lewis et al. 1992), which have shown that vitrectomy with removal of the posterior hyaloid may be beneficial in eyes with diffuse DME associated with a thickened, taut premacular posterior hyaloid, there has been an interest in vitreous surgery as a potential treatment for DME. Many investigators have reported that vitrectomy is beneficial for DME, especially for eyes with DME and a thickened, taut posterior hyaloid (Harbour et al. 1996; Pendergast 1998; Ikeda et al. 1999; Sato et al. 2002; Kalvodová et al. 2002, Yamamoto et al. 2003, Aboutable et al. 2005). Macular edema was hypothesized to exacerbate by tangential traction of the thickened and still attached posterior hyaloid membrane, causing a

very shallow macular detachment similar to that observed in patients with macular holes (Lewis et al. 1992; Harbour et al. 1996; Pendergast 1998; Pendergast et al. 2000). Others have found that even among patients whose DME is not accompanied by visible evidence of posterior hyaloid thickening or traction some respond to vitrectomy with resolved DME and improved vision (Tachi et al. 1996, Ikeda et al. 1999, Otani et al. 2000, Ikeda et al. 2000; La Heij et al. 2001, Yamamoto et al.2001, Aboutable 2006). If vitrectomy is proved to be beneficial for DME, it could have a major impact on the quality of life of numerous diabetic patients. Since first report on resolution of DME after surgical removal of the posterior hyaloid and the internal limiting membrane (ILM) (Gandorfer et al. 2000), little has been published on ILM peeling for DME (Avci et al. 2004, Kuhn et al. 2004, Dillinger et al. 2004, Aboutable 2006), some investigators reported resolution of macular edema and improvement of VA (Avci et al. 2004, Kuhn et al. 2004) others found that the ILM peeling accelerates the absorption of edema in more sever diabetic cases, without any improvement of VA (Kumagai et al. 2002).Within last four years, intravitreal application of triamcinolone acetonide (IVT) has increasingly used as a treatment option for DME (Jonas and Söfker 2001, Martidis et al. 2002, Massin et al. 2004, Chieh et al. 2005). IVT allows extremely high concentrations of steroid at its site of acquired action, and simultaneously decreases or avoid systemic side effects. Triamcinolone acetonide (TA) can be applied (1) into the vitreous body as an intravitreal injection, (2) as a subtenon injection, (3) or into the vitreous cavity at the end of vitrectomy.

2. STUDY PURPOSE

The aims of this study are to determine the effectiveness of vitrectomy as a treatment option for DME, the possible role of additional ILM peeling during vitrectomy and to investigate safety and efficacy of IVT as an adjunctive treatment injected into the vitreous cavity at the end of vitrectomy. The study includes 4 study groups:

- First study (vitrectomy study): The purpose of this study is to evaluate anatomic and functional results of vitrectomy for DME refractory to laser treatment in eyes with different states of posterior vitreous membrane and different duration of the edema. To report intraoperative and postoperative complications.
- Second study (ILM peeling study): The aim of this controlled study is to evaluate the effect of the ILM peeling during vitrectomy in eyes with DME without evident epimacular proliferation or cellophane maculopathy, unresponsive to laser photocoagulation. To determine whether ILM peeling improves anatomical and functional outcomes and whether is always essential in DME surgery.
- Third study (triamcinolone study): The purpose of this ongoing study is to determine whether application of IVT at the end of vitrectomy is safe and effective in treating DME refractory to prior laser photocoagulation.
- Fourth study (histopathological study): The aim of this study is to describe the histopathological features of the ILM intentionally removed during vitrectomy for DME and to compare them with those peeled during vitrectomy for idiopathic macular hole (MH).

3. METHODS

3.1 STUDY GROUPS AND DESIGN

All surgeries were performed at the Department of Ophthalmology, General Faculty Hospital and 1st Faculty of Medicine, Charles University of Prague between 2001 and 2006.

All patients received full information regarding all available treatment options and all of them gave their informed consent prior to the surgery. All studies were performed in a prospective design and included the following numbers of eyes:

- First study (*vitrectomy study*): This study includes 72 eyes (61 patients). All surgeries were performed between June 2001 and December 2003.
- Second study (*ILM peeling study*): In this controlled study, ten patients (20 eyes) with similar degree and duration of DME in both eyes underwent bilateral vitrectomy with and without ILM peeling to determine the role of ILM peeling in DME surgery. All patients were operated between April 2003 and January 2005.
- Third study (*triamcinolone study*): This ongoing study started in January 2005. At the end of July 2005 the study included 32 eyes (32 patients).
- Forth study (*histopathological study*): This comparative, interventional case series, using transmission electron microscopy study includes 6 samples of ILM obtained from 6 eyes (6 patients) during vitrectomy performed between September 2005 and January 2006. The histopathological analysis was performed by Assoc. Prof. Jaroslava Dušková M.D., CSs. at the Department of Pathology, the 1st Faculty of Medicine, Charles University in Prague.

All patients from the first and the second studies were followed up for at least 6 months after surgery. Patients from the third study were followed up at least 4 months.

3.2 INCLUSION AND EXCLUSION CRITERIA

3. 2. 1. The first and the third studies (the vitrectomy study and the triamcinolone study)

Inclusion criteria:
(1) Clinically significant diffuse DME with cystoid changes.
(2) Refractory to prior macular laser photocoagulation.

Excluded were eyes with:
(1) Ophthalmic disorders associated with macular edema, such as uveitis and branch or central retinal vein occlusion.
(2) Fibrovascular proliferation with tractional and/or rhegmatogenous retinal detachment and/or macular distortion.
(3) Dens media opacity such as cataract or vitreous hemorrhage.
(4) History of pervious vitrectomy.

3. 2. 2. The second study (the ILM peeling study)

Inclusion criteria for this study were as follows:
(1) Patients with clinically detectable bilateral DME with similar degree and duration of DME in both eyes (visual disparity between the both eyes was less than 3 lines).
(2) Refractory to laser photocoagulation.
(3) No evident epimacular proliferation or cellophane maculopathy.
(4) Posterior hyaloid attachment without evident vitreomacular traction.

Excluded were eyes with:
(1) Ophthalmic disorders associated with macular edema, such as uveitis and branch or central retinal vein occlusion.
(2) Dens media opacity such as cataract or vitreous hemorrhage.
(3) Fibrovascular proliferation with tractional retinal detachment and/or macular distortion.
(4) History of pervious vitrectomy.

3. 2. 3. The fourth study (histopathological study)

Inclusion criteria:
Eyes with refractory diffuse DME or idiopathic macular hole that underwent vitrectomy with ILM peeling, in which removed ILM were >2 disc diameters (PD).

Excluded were eyes in which the peeled ILM were < 2 PD in diameter or were destroyed during fixation, therefore theirs excellent histopathological analysis were not possible.

3. 3 PREOPERATIVE COLLECTION DATA

The following preoperative data were recorded for each patient from any of the 4 studies:

- Age, gender, type and duration of diabetes, glycaemia, HbA1c in most patients, history of arterial hypertension and/or nephropathy.
- Severity of diabetic retinopathy, pervious ocular surgery, history of focal, grid and panretinal laser photocoagulation.
- The duration of DME was defined as the interval between the time at which the patient was first seen in our clinic with DME and the time of operation. According to the duration of DME all eyes from the first study were divided into two groups, eyes with duration of DME shorter than 6 months and eyes with duration of DME longer than 6 months.
- Best-corrected Snellen visual acuity (BCVA), visual acuity for near and intraocular pressure by applanation tonometer.
- Careful examination of the anterior segment by the slit-lamp biomicroscopy to evaluate the absence or presence of neovascularization of the iris, lens status and to determine whether the view will be adequate to perform the precise surgical procedures that are required.
- The diagnosis of DME was made by careful ophthalmoscopy. Biomicroscopic examination of the posterior pole, with contact and noncontact (90 diopters or 78 diopters) fundus lenses, was performed to verify the presence of DME, to evaluate the vitreoretinal interface relationships and to check for any preretinal membranes. To determine the stat of the posterior hyaloid membrane, such as attachment or detachment of the posterior hyaloid and whether the still-attached posterior hyaloid was thickened.
- Fluorescein angiography and was performed in most cases to determine the retinal leakage and the capillary perfusion.
- Standardized sonography was performed as a routine examination for all patients to document the status of the posterior hyaloid membrane and the presence of any vitreoretinal traction on the macula.
- Stratus OCT, Carl Zeiss 2. and 3. versions were used.

3. 4 SURGICAL TECHNIQUES

All operations in the first study were performed by two surgeons (BK, TA), in the second study by one surgeon (TA), in the third study by three surgeons (BK, TA, JD) [*].All surgeries were performed under local anaesthesia by using 5 ml Bubivacaine 0.5% injected into the retrobulbar space. Our vitrectomy technique consisted of a standard three-port vitrectomy. Our usual vitrectomy settings are 600-800 cuts per minute and suction levels of 200-250 mmHg. Intraoperatively, special attention was paid to the vitreoretinal interface: whether the posterior hyaloid membrane was attached or partially or complete detached, whether it was abnormally thickened, whether there was an epiretinal membrane, and whether there was a cellophane-type glistening with or without wrinkling of the inner retinal surface

([*]BK: Assoc. Prof. Bohdana Kalvodová M.D., CSc, TA: Tarek Aboutable M.D., JD: Jan Dvo ák M.D.)

at the level of the ILM. In eyes with posterior hyaloid attachment the posterior hyaloid was detached from the posterior pole by using high suction power with the vitrectomy instrument at the optic disk until detachment was created, as evident by a Weiss ring. Further separation of the hyaloid towards the peripheral retina was achieved with the vitrectomy instrument, using a combination of suction and cutting. Sixty one (61) eyes from the first study underwent vitrectomy without ILM peeling and 11 eyes that showed an epimacular proliferation and a cellophane maculopathy, underwent vitrectomy with ILM. Ten eyes from the second study underwent vitrectomy without ILM peeling and 10 eyes underwent vitrectomy with ILM peeling. Eyes that underwent vitrectomy with ILM peeling in the second study were selected at random. ILM was stained with about 0, 3 ml of Trypan-blue 0.2% (Membrane Blue, D.O.R.C. International, Zuidland, The Netherlands) injected in the front of the macula in a liquid-filled eye. The dye was removed in 60 s. Afterwards the ILM was incised and peeled within the temporal vascular arcade using forceps. Intraoperative focal laser photocoagulation for proliferative DR (PDR) was necessary in 30 eyes from the first group; eight eyes from the second study with PDR also received focal laser photocoagulation. No grid or additional focal macular laser therapy was applied intraoperatively. Endotamponade (20% sulphur hexafluoride) was used in one complicated eye from the first study, in one complicated eye from the second study and in 2 eyes from the fourth study. We carefully checked with scleral indentation the retinal periphery for iatrogenic tears. At the end of surgery, subconjunctival injections of dexamethasone (2mg) and gentamycin (4 mg) were administered. Postoperatively, eyes were treated with gentamycin 3 mg drops for 1 week, dexamethasone 1 mg drops and atropine 1% drops for approximately 1 month.
In the third study (*triamcinolone study*) we injected 4 mg (0.1 ml x 40 mg) of TA into the eye at the end of vitrectomy with removal of the posterior hyaloid. The following method was routinely used to isolate TA particles and to remove preservatives and suspending agents in the vehicle (benzyl alcohol, carboxymethylcellulose) from its commercially available suspension before intravitreal application as previously described in detail (Jonas and Sofker 2001):

- The contents of a Triam vial (40 mg TA suspended in 1.0 ml vehicle) were loaded into a syringe with a three-port valve and passed through a millipore filter (pore size 5 µm; Sterifix Pury, B Braun Melsungen AG, Carl-Braun-Strasse 1, 34212 Melsungen, Germany).
- The filter was then backflushed with Ringer's solution to yield a vehicle-poor suspension of TA in the initial syringe.
- This filtration and backflush procedure was repeated four times.
- The staff of the operating theatre before the intravitreally application routinely performed the filtration procedure.

3. 5 POSTOPERATIVE COLLECTION DATA

Postoperatively collected data included:

- BCVA and intraocular pressure by applanation tonometer.
- Careful examination of the anterior segment by the slit-lamp biomicroscopy.
- Absorption, presence or recurrence of DME detected by careful biomicroscopic examination of the posterior pole performed 1 week, 1, 3 and 6 months after surgery.
- Length of follow-up period.
- Any postoperative complications.
- Postoperatively controlled OCT was performed 6 months after surgery in most cases of the first study, in all cases of the second study and in a few cases of the third study. The status of DME after surgery in the third study was largely based on slit-lamp ophthalmoscopy. Wilcoxon Rank Sum Test and Wilcoxon Signed Rank Test were used for statistical analysis in the second study.

3. 6 HISTOPATHOLOGIC EXAMINATION

Morphometric Evaluation of the ILM in the LUCIA G5 (Laboratory Universal Computer Image Analysis) Image Analysis System.

1) 2.5% glutaraldehyde fixed surgically peeled ILM and was embedded into artificial resin (Durcupan- Epon).
2) Semithin sections were stained with toluidine blue and ILM identified.
3) Ultrathin sections were contrasted with uranyl acetate and lead citrate.
4) ILM was photographed with the Jeol 100SX transmission electron microscope under the standard enlargement 5000x with a 1µm scale.
5) In the LUCIA G5 (Laboratory Imaging, Prague) the digitalized images were loaded, superimposed with a square grid of 500 px (= 3.25 µm). Any hit of the grid on a membrane was a place for the transversal ILM thickness measurement. The ILM of any patient with sufficient length of the membrane provided was measured on 10 photographs providing thus 40-50 dimensions for subsequent arithmetic mean +SD evaluation.
6) MS Excel predefined table served the final Arithmetic Mean + SD evaluation.

4. RESULTS

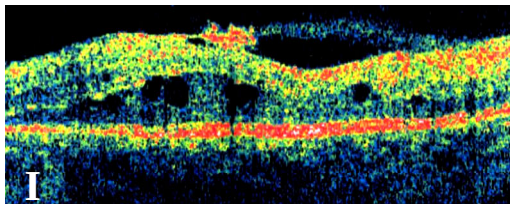
4. 1. FIRST STUDY

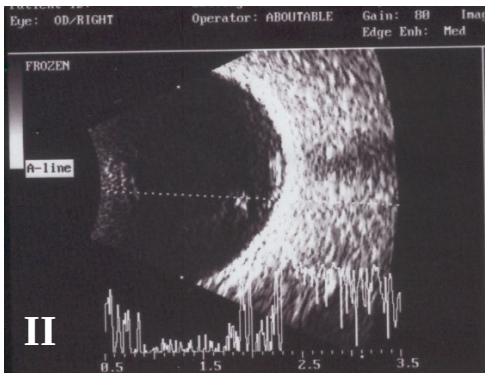
Seventy two consecutive eyes of 61 patients were included in the study, 26 (42, 6%) were females and 35 (57, 4%) were males. The right eye was operated on in 55%. Thirty (41, 7%) eyes with PDR were treated by panretinal laser photocoagulation and 42 (58, 3%) with NPDR received focal laser therapy.

According to the status of the posterior vitreous membrane examined by careful biomicroscopic examination and sonography all eyes were divided into 3 groups:

- Group A: 21 (29, 2%) eyes with attached and taut premacular posterior hyaloid with evident vitreomacular traction (**Fig. 3, 4, 6**).
- Group B: 36 (50%) eyes with attached posterior hyaloid in the macular region, but without thickening and without traction on the macula (**Fig. 5**)
- Group C: 15 (20, 8%) eyes with posterior vitreous detachment.

Figure 3. I, II, III:
Patient (from group A) with attached, taut and thickened posterior hyaloid causing tangential vitreomacular traction





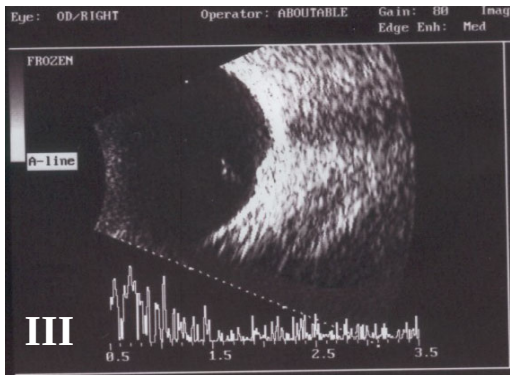


Figure 4. CME with partial detachment of the posterior hyaloid causing anterioposterior vitreomacular traction

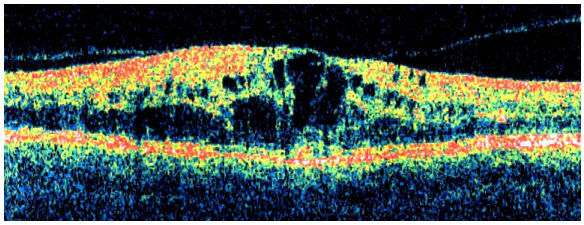
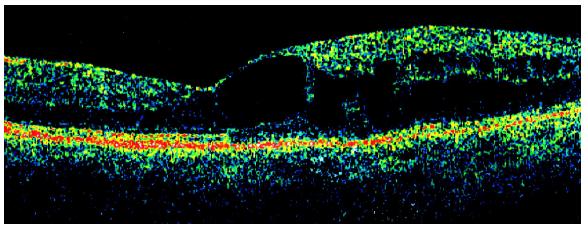


Figure 5. Attached posterior hyaloid in the macular region, without thickening, and without traction on the macula



Forty four eyes with duration of DME shorter than 6 months (15 eyes from group A, 20 eyes from group B, 9 eyes from group C) and 28 eyes with duration of DME longer than 6 months (6 eyes from group A, 16 eyes from group B, 6 eyes from group C). Cystoid changes were observed as diagnosed by biomicroscopic examination and confirmed by fluorescein angiography and/or OCT in all eyes. The median duration of the macular edema was approximately 9.0 months (range 2-32 months) at the time of vitrectomy. The preoperative visual acuities ranged from 0.02 to 0.4(median 0.2, mean 0.25). ILM peeling was performed in 11 eyes from group C.

Table 1 Anatomic results

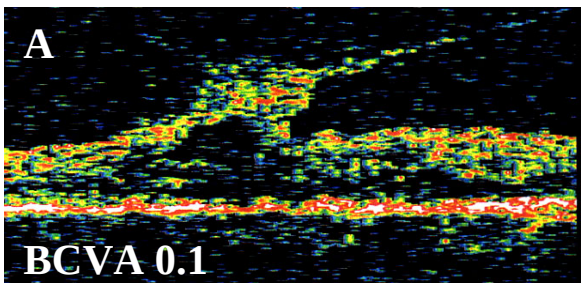
DME	**Group A (*n* =21)**	**Group B (*n* =36)**	**Group C (*n* =15)**
Resolved	20(95%)	21(58%)	3(20%)
Decreased	1(5%)	14(39%)	10(66%)
Unchanged	–	1(3%)	1(7%)
Increased	–	–	1(7%)
Recurrence	–	–	–

Table 2 Functional results

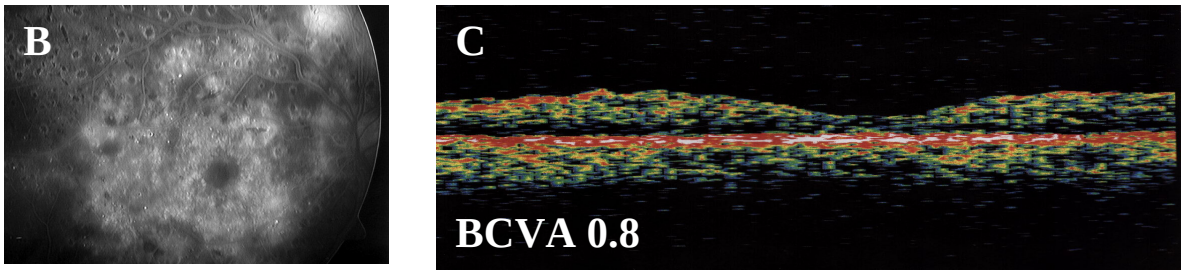
BCVA	**Group A (*n* =21)**	**Group B (*n* =36)**	**Group C (*n* =15)**
Increased [2 lines]	19(90%)	23(64%)	3(20%)
Unchanged	2(10%)	11(30%)	9(60%)
Deteriorated	–	2(6%)	3(20%)

Edema resolved completely 44 eyes (61%), decreased in 25 eyes (35%) (**Tab. 1**), the final visual acuity improved by 2 or more lines in 45 eyes (63%), remained unchanged in 22 eyes (31%), exacerbated after surgery in 5 eyes (7%) (**Tab. 2**), due to residual CME, massive macular hard exudates, and iatrogenic macular hole. Among eyes with visual acuity improvement by 2 or more lines (total 45 eyes) 37 eyes had duration of DME shorter than six months (15 eyes from group A, 19 eyes from group B, 3 eyes from group C) and 8 eyes had duration of DME longer than 6 months (4 eyes from group A, 4 eyes from group B). The average follow-up time was 14 months (range 6 - 27 months).

Figure 6.
A. Preoperative vitreomacular traction by the adherent posterior hyaloid following a partial posterior vitreous detachment results in retinal vascular leakage (**B**) in a patient with preoper-operative BCVA 0.1



C. 2 months after vitrectomy the BCVA was 0.8



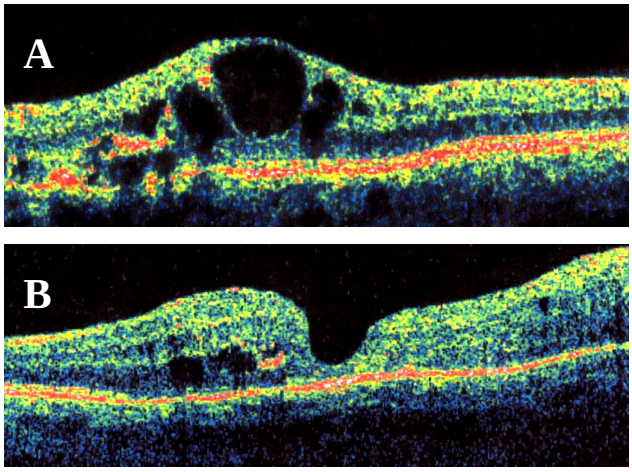
Complications during surgery included:

- Peripheral retinal tear formation in one eye (1, 4%), could be treated by endolaser photocoagulation.
- Postoperatively easy vitreous haemorrhage was found in two eyes (2, 8%) and was resolved within 1 week.
- Cataract formation observed in two eyes (2, 8%).

We noticed that peeled ILM were thickened and showed great adherence to the retina, therefore theirs peeling was usually more difficult than in eyes with macular hole and takes more time because of its tendency to tear, specially in eyes with larger cysts. Among 11 eyes, that underwent ILM peeling, cyst rupture with formation of macular hole was documented in one eye (9%) with large cystoid spaces composed of thin inner retinal layer (**Fig. 7**). Required a fluid-gas (20% sulphur hexafluoride) exchange and the patient was asked to remain in a face- down position until gas absorption (14 days), then the iatrogenic macular hole was closed. Neither epiretinal membrane nor recurrence of the macular edema was documented.

Figure 7.
A. Preoperative large cystoid spaces composed of thin inner retinal layer

B. Postoperative decrease in the macular thickness and closure of the iatrogenic macular hole



4.2. SECOND STUDY

Ten patients (6 men and 4 women; 20 consecutive eyes), aged 45 to 62 years (average 56) made up the study population. The median duration of the edema was approximately 12.0 months (range 6-21 months) at the time of the surgery. The average follow-up time was 13, 6 months (range 6 - 21 months). As diagnosed by biomicroscopic examination, sonography and OCT the posterior hyaloid was found to be attached in all eyes, but no taut, rigid membrane was found in any eye. OCT showed retinal swelling in all 20 eyes. An area of low reflectivity was mainly located in the outer retina. In addition to retinal swelling, cystoid spaces were observed in 18 of 20 eyes, and a serous retinal detachment in two eyes.

Eight (40%) eyes with proliferative diabetic retinopathy were treated preoperatively by panretinal laser photocoagulation and 12 (60%) eyes with non-proliferative diabetic retinopathy received focal laser therapy. All eyes were treated unsuccessfully preoperatively by focal macular laser; no grid laser was performed because of its limited beneficial effect in the treatment of diffuse or cystoid type of DME. Baseline BCVA and foveal thickness ranged, respectively, from 0.4 to 0.05 (mean 0.18) and 430 to 840 µm (mean 618) in eyes that underwent ILM peeling, 0.5 to 0.05 (mean 0.16) and 390 to 910 µm (mean 623 µm) in eyes without ILM peeling. There were no significant differences between the both groups in baseline BCVA (P = 0.4691, Wilcoxon Rank Sum Test) or foveal thickness (P= 0.8204, Wilcoxon Rank Sum Test). At six- months follow-up, mean BCVA improved significantly in both groups, from 0.18 to 0.33 (P = 0.0427, Wilcoxon Signed Rank Test) in eyes that underwent ILM peeling (**Fig. 8**) and from 0.16 to 0.25 (P= 0.0482, Wilcoxon Signed Rank Test) in eyes without ILM peeling. Mean foveal thickness decreased significantly from 618 to 265 (P= 0.0050) in eyes with ILM peeling and from 623 to 311 (P= 0.0050) in eyes without ILM peeling. Visual acuity improved by two or more lines in five eyes (50%) of each group. There were no significant differences in the improvement of BCVA and decreasing of foveal thickness between the both groups (Wilcoxon Rank Sum Test, P = 0.9083, P = 0.2720, respectively).

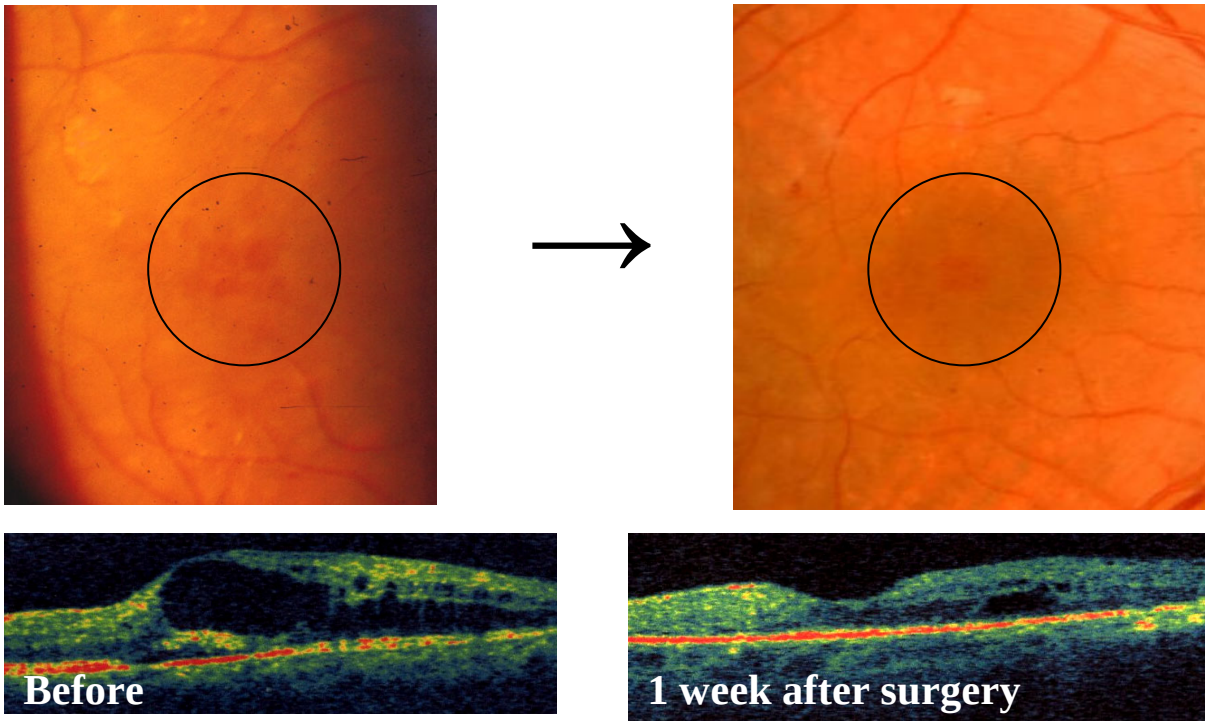


Figure 8. 1 week after vitrectomy with ILM peeling: The foveal thickness decreased from 560 µm to 220 µm; most cystoid spaces disappeared (note the cystoid spaces inside the circle before the surgery); the foveal depression was observed (note the circle after surgery); and the BCVA improved by 4 Snellen lines.

Complications during surgery included peripheral retinal tear formation in one eye (5%) could be treated with laser photocoagulation and cyst broke during peeling ILM in one eye (5%) with formation of macular hole in a case with large cystoid spaces composed of thin inner retinal layer. Required a fluid-gas (20% sulphur hexafluoride) exchange and the patient was asked to remain in a face- down position until gas absorption. We noticed that peeled ILM were thickened and showed great adherence to the retina, therefore theirs peeling was usually

more difficult than in eyes with macular hole and takes more time because of its tendency to tear, specially in cystoid macular edema with bigger cysts. Neither epiretinal membrane nor recurrence of the macular edema was found during the follow-up period.

4.3. THIRD STUDY

Thirty tow consecutive eyes of 32 patients with median age 59.54 years were included in the study. Eight (15 %) eyes with PDR were treated preoperatively by panretinal laser photocoagulation and 24 (75%) with NPDR received focal laser therapy. The mean baseline foveal thickness was 497.4 µm. The baseline BCVA ranged from 0.08 to 0.63 (mean 0.212). The mean follow-up time was 5.5 months (range 4 - 7 months). BCVA improved by 2 lines in 8 eyes (25%), stabilized in 20 eyes (62.5 %) and deteriorated in 4 eyes (12.5%). The foveal thickness decreased postoperatively in 23 eyes (71.8%) (**Fig 9**). Early postoperative complications included:

- Elevation of the intraocular pressure (< 35 mm Hg) in 7 eyes (21. 87%), all eyes could be treated by using local antiglaucoma medication within 1 month postoperatively.
- Retinal detachment in one eye required additional peripheral vitrectomy with fluid-gas (16 % C3F8) exchange.
- Acute bacterial endophthalmitis (Staphylococcus epidermidis) in one eye required additional vitrectomy with intravitreal application of antibiotics and injection of silicon oil.

During the fellow-up period cataract formation was reported in 2 eyes.

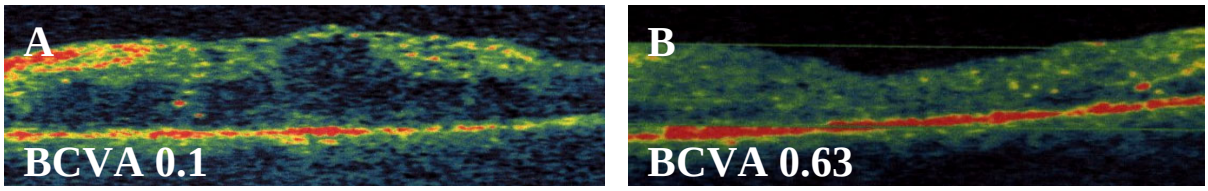


Figure 9. A: Before surgery **B**: 1 week after vitrectomy and application of 4 mg of triamcinolone acetonide

4.4. FORTH STUDY

Four eyes with DME (DME group) and 2 eyes with idiopathic macular hole (MH group) were studied (**Tab 3**.) The DME group consisted of three men and one woman, with a mean age of 58.5 years (ranging from 52 to 65 years). The mean period from the diagnosis of DME to the surgery was 7.5 months. All eyes in the DME group were refractory to laser photocoagulation therapy performed at least 3 months before the surgery. The MH group consisted of one man and one woman with a mean age of 53 years. The both eyes were classified as stage 3 MH (Gass 1995). The mean period from detection of MH to ILM peeling was 3 months. In the ultrastructural examination, all surgical specimens were demonstrated to be ILM tissues. Transmission electron microscopy revealed ILM in the both groups as a membrane with a smooth inner surface and an irregular undulating outer surface. The ILM in the both groups were composed of homogeneous electron-dense meshwork and these ultrastructural findings of the ILM correspond in shape to the lamina dense of basement membrane. The mean thickness of the peeled ILM was 3.77 µm (SD 0.71) in the DME group and 3.58 µm (SD 1.74) in the MH group (**Fig 10**). A few cellular elements seem to be macrophages were observed only on the vitreous side of ILM in the both groups. No structural or morphological differences of the specimens were observed between the two groups.

Table 3. Preoperative patient's data and thickness of the peeled ILM

Patient number	Age/Sex	Surgical diagnosis	Duration of DME or MH (months)	Mean Thickness of ILM in micrometers
1	52/F	DME	6	2.57 (SD 0.49)
2	61/M	DME	7	3.74 (SD 0.88)
3	56/M	DME	10	4.11 (SD 1.18)
4	65/M	DME	7	4.69 (SD 0.30)
5	51/M	MH	2	2.67 (SD 1.61)
6	55/F	MH	4	4.49 (SD 1.88)

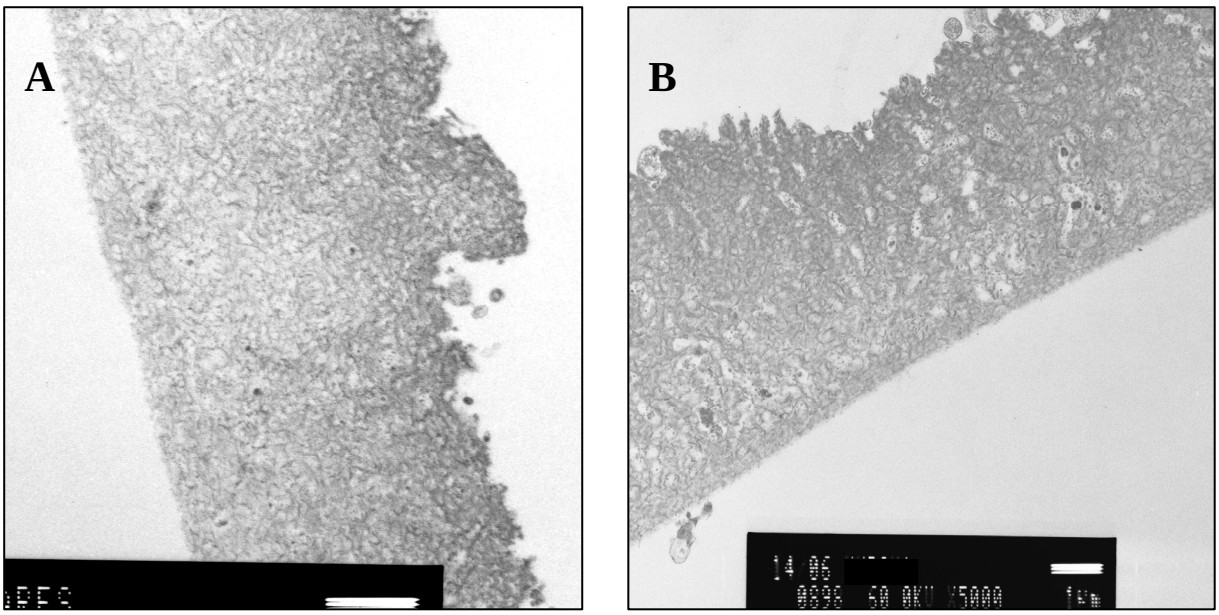


Figure 10. A: ILM removed from patient with DME, ILM thickness is 4.11 µm (SD 1.18). **B**: ILM removed from patient with MH, ILM thickness is 4.4 µm (SD 1.8). Normal thickness of the ILM is about 0.5 µm (Williams and Warwick 1980).

5. DISCUSSION

Many systemic abnormalities may affect macular thickness (Bresnick 1986, Stratton et al. 2001); therefore the treatment of DME includes medical control of these systemic abnormalities. Patients should achieve excellent glycemic control, normalize blood pressure, improve cardiac and renal status, and reduce serum lipids.

The ETDRS has shown that focal laser photocoagulation of leaking circumscribed retinal areas in eyes with focal DME is therapeutically useful to improve visual outcome. In eyes with diffuse DME, however laser treatment cannot be focused on localized retinal leakage spots since the entire macula is involved. Diffuse DME is, therefore, much less responsive to macular laser coagulation than focal DME (Olk 1986, Lee and Olk 1991). In ETDRS laser photocoagulation therapy reduced the risk of moderate visual loss by 50%, the improvement rate was below 3%, and 12% eyes had significant visual loss for patients with clinical significant DME at 3 years (ETDRS 1985, ETDRS 1987).

Since 1990, several authors have reported favourable anatomical and satisfactory functional results in-patients with DME undergoing vitrectomy combined with removal of the posterior hyaloid and premacular hyaloid –associated traction forces. Other investigators have

reported that vitrectomy may be useful, even in the absence of obvious posterior hyaloid anomalies (Tachi et al. 1996, Ikeda et al. 1999, La Heij et al. 2001, Otani et al. 2000, Yamamoto et al. 2001, Aboutable et al. 2005) and even when the posterior hyaloid was detached from the posterior pole (Ikeda et al. 2000). Several explanations have been suggested for postvitrectomy improvement of DME in the absence of vitreomacular traction. The vitreous may act as a potential reservoir of inflammatory substances or growth factors such as vascular endothelial growth factor, witch promotes vascular permeability (Aiello 1997), and its removal by vitrectomy may improve DME. Another possible explanation is that vitrectomy may improve oxygenation of the retina (Stefansson et al. 1990). The results of our first study (the vitrectomy study) support the effectiveness of vitrectomy in resolving DME as first reported by Lewis et al. in 1992 and later studies by others (**Tab. 7**). We found that vitrectomy is effective in eyes with taut posterior hyaloid and found among patients whose DME is not accompanied by visible evidence of posterior hyaloid thickening or traction some respond to vitrectomy with resolved DME and improved vision. We found vitrectomy to help in reducing the DME in majority of eyes. This however, is not always associated with VA improvement. Although this study is not controlled, we suggest that vitrectomy for DME seems to be more effective than traditional management of observation or further therapy by laser photocoagulation. In this study better outcomes were achieved in eyes with evident vitreomacular traction and with short duration of the edema. In agreement with earlier study by Harbour et al. (Harbour et al. 1996) we found that a shorter time interval from initial diagnosis of macular edema to vitrectomy may be associated with a better visual outcome. This discrepancy between anatomic and functional results may relate to irreversible destructive changes of the macula due to long- standing DME on the retinal layers (Gass 1987). This may suggest that early surgical intervention may improve the chances of visual recovery when performed before the occurrence of severe visual loss, therefore optimal timing of the surgery seems to be important. These surgical results have made us considerably reduce the use of laser photocoagulation for DME and decide for earlier intervention in selective cases.

Table 7 Results of vitrectomy for DME in some previous studies

		No (%)		
Study	No. of eyes	Resolved DME	BCVA improvement 2 lines	Follow-up (months)
Lewis et al. 1992	10	8(80)	6(60)	16
Van Effenterre et al.1993	22	12(45)	19(86)	14
Harbour et al. 1996	7	4(57)	4(57)	12
Pendergast et al. 2000	55	45(82)	27(49)	23
La Heij et al. 2000	21	21(100)	10(47)	10.2
Kalvodová et al. 2002	10	10(100)	6(60)	9
Yamamoto et al. 2003	65	30(46)	29(45)	12.6
Aboutable and Kalvodová 2005	72	69(96)	45(63)	14

The primary results of our first study were presented at the X. Annual Meeting of the Czech Society of Ophthalmology in Hradec Králové (2003), and the final results were presented at the 102nd Annual Meeting of the German Society of Ophthalmology in Berlin (September 2004) and have been published in Klin Monatsbl Augenheilkd. 2005 Aug; 222(8): 643-8.

The innermost layer of the retina, the ILM, is composed in part of type IV. collagen and its normal thickness is about 0.5 μm (Williams and Warwick 1980). This transparent structure rests on a bed of Müller cell footplates that in turn from a contiguous montage, separating the ILM from the nerve fibre layer (Williams and Warwick 1980, Kuhn 2002). The

ILM received virtually no clinical attention until vitrectomy removal of epimacular proliferation become routine in the 1980s and ILM fragments were often identified in surgical specimens. First study about the possible role of ILM peeling for DME reported resolution of the DME after surgery in 12 eyes. In this study however, a thickened posterior hyaloid membrane was removal in the same session in 10 of the 12 eyes (Gandorfer et al. 2000), and therefore no useful conclusion on the effectiveness of ILM peeling alone can be drawn from this study. How additional ILM peeling may reduces DME is unclear. The discussed mechanisms are complete releasing of the tractional forces on the macula and inhabitation of reproliferation of fibrous astrocyts (Gandorfer et al. 2000, Radetzky et al. 2004) by removing the template (the ILM) on which the glial tissue proliferates and contracts. Histopathologic studies in MH surgery have supported the supposition that redirection of glial proliferation is the mechanism by which macular hole closure is effected (Funta et al. 1992, Madreperla et al. 1994, Rosa et al. 1996). Indeed, because ILM is composed of the footplates of Müller cell, we suggest that shearing the cells may injure the cells and thus represent the specific stimulus for glial proliferation, which may play a role in reducing the macular edema and stimulate resynapses between the bipolar cells and the ganglion cells in the inner plexiform layer. Tano (Tano 2000) has reported that ILM taken from DME cases were indeed almost twice as thick as the ILM taken from MH cases; therefore it was suggested that abnormally thickened ILM might play a role as a diffusion barrier for the vitreous cytokines such as vascular endothelial growth factor (VEGF) and others leading to capillary permeability disturbance (Connolly 1991, Collins et al. 1993, Miller et al. 1994, Hofman et al. 2001). It was suggested that ILM peeling dose not influence the pathophysiological changes such as growth factor expression or altered fluid dynamics but it is much more likely that ILM peeling merely reduces the diffusion barrier towards the vitreous and thus is more efficient in patients with preexisting interface alterations. The improvement after surgery is just as likely to be attributable to the vitrectomy as to the ILM peeling (Radetzky et al. 2004). To date little has been published on ILM peeling for DME. Some investigators provided ILM peeling generally in cases with DME (Gandorfer et al. 2000, Avci et al. 2004, Dillinger et al. 2004, Kuhn et al. 2004), others only in eyes that showed visible epimacular proliferation and cellophane maculopathy (La Heij et al. 2001, Aboutable et al. 2005). Some investigators reported on resolution of macular edema and improvement of VA (Avci et al. 2004, Dillinger et al. 2004, Kuhn et al. 2004), others found that the ILM peeling accelerates the absorption of edema in more sever diabetic cases, without any improvement of VA (Kumagai et al. 2002). In a recent retrospective study DME resolved and VA improved in eyes after vitrectomy without ILM peeling in 44, 4% and in 69, 1% of eyes that underwent vitrectomy with ILM peeling (Stefaniotou et al.). Pervious studies about ILM peeling for DME were not controlled and no firm conclusions can be drawn, whether ILM peeling for DME should be indicated generally as a standard surgical procedure or selectively in eyes with visible epimacular proliferation and cellophane maculopathy.

Our second study (the ILM peeling study) is certainly limited by the small number of patients included. However, this is the first controlled study of ILM peeling for DME without evident epimacular proliferation or cellophane maculopathy. Systemic conditions such as glycaemia, blood pressure and nephropathy differ in each case and may affect the surgery results. Because we used in the second study the fellow eyes as controls it was not necessary to consider the difference of individual systemic condition. The findings of the current study demonstrated that in eyes with persistent DME without evident epimacular proliferation vitrectomy without ILM peeling was as effective in reducing the foveal thickness and improving the visual acuity as vitrectomy with ILM peeling. There were no significant differences in the improvement of BCVA and decreasing of foveal thickness between the both groups. We conclude that peeling of the ILM is not essential for anatomic and visual success

in DME surgery. Our results of the second study were presented at the 15th Congress of the European Society of Ophthalmology and 103rd Annual Meeting of the German Society of Ophthalmology in September 2005 in Berlin, at the 5th Meeting of the Czech Vitreoretinal Society in November 2005 in Prague, and accepted for publication in Klin Monatsbl Augenheilkd. Shortly after the presentation of our results in Berlin, Yamamoto and associates (Yamamoto et al. 2005) published the results of their prospective, controlled study about ILM peeling for DME and concluded that ILM need not to be removed to treat eyes with ILM. Yamamoto's results support the findings of our pilot controlled study.
In MH surgery, ILM peeling has been proven to be a feasible and safe procedure that provides favourable anatomical and functional results (Brooks 2000). ILM peeling in eyes with DME, compared to eyes with macular hole was more difficult; ILM was more adherent to the retina especially in eyes with larger cysts. Cyst rupture during ILM peeling with formation of iatrogenic macular hole is a serious complication, which should be included in the list of the complications of ILM peeling in eyes with fragile edematous retina; therefore maximal attention should be paid for ILM peeling in these conditions.

Several complications have been reported after vitrectomy with or without ILM peeling. These complications included:

- The appearance of a dissociated optic nerve fiber layer characterized by numerous arcuate striae within the posterior pole described by Tadayoni et al. (Tadayoni et al. 2001), the retina along the course of optic nerve fibers was slightly darker than the surrounding retina on blue-filter photographs. They reported that this appearance was detected in 43% of eyes that had undergone epiretinal membrane peeling and was also observed after ILM peeling during macular hole surgery. Although the pathogenesis of this phenomenon is not known, they suggested that it could be due to permanent damage to the Müller cells.
- Intraoperative retinal tears and postoperative rhegmatogenous retinal detachment (Tachi et al. 1996, Gandorfer et al. 2000, Lewis et al.1992). This complication has been documented in our study. Creating of posterior vitreous detachment can be associated with peripheral retinal tears; therefore we found controlling the retinal periphery by scleral indentation at the end of vitrectomy very important. Using the exocryocoagulation or endolaser photocoagulation the iatrogenic tears can be successfully treated.
- Postoperative vitreous haemorrhage (Gandorfer et al. 2000, Lewis et al.1992).
- Neovascular glaucoma (Tachi et al. 1996).
- Cataract formation (Tachi et al. 1996, Gandorfer et al. 2000, Lewis et al.1992).
- Macular ischemia (Harbour et al. 1996).
- A lamellar macular hole associated with vitrectomy and removal of an epiretinal membrane without ILM peeling (Yamamoto et al. 2003).
- A cyst rupture with an iatrogenic macular hole associated with ILM peeling was documented in a case with large cystoid spaces (Yoon et al. 2003). This serious complication has been reported also in our first and second studies in two cases with large cystoid spaces composed of thin inner retinal layer. Required a fluid-gas (20% sulphur hexafluoride) exchange and the patient was asked to remain in a face- down position until gas absorption. We found that the performing of the ILM peeling in such cases include a high risk of cyst rupture, therefore ILM peeling showed be indicated very carefully. Generally, in all eyes with cystoid DME extreme caution should be paid during ILM peeling to avoid cyst rupture and iatrogenic macular hole.
- Pendergast et al. observed postoperative epiretinal formation after removal of posterior hyaloid without ILM peeling for DME in 6 of 59 eyes (Pendergast et al. 2000); also Tachi (Tachi et al. 1996) reported this postoperatively complication in 6 from 58 eyes. This complication was not observed in the study by Gandorfer et al. (Gandorfer et al. 2000) after additional ILM peeling.

- Pendergast et al. reported recurrence of DME after ILM peeling in 3 of 55 eyes (Pendergast et al. 2000); others did not document this complication (Gandorfer et al. 2000). In our first and second studies neither epiretinal membrane nor recurrence of the DME was reported during the follow-up period, in the third study also these complications were not found during the short follow-up time.

TA is a corticosteroid suspension that has been used locally as a periocular injection for the treatment of cystoid macular edema secondary to uveitis or as a result of intraocular surgery (Stern et al. 1981, Suckling et al. 1988). Intravitreal corticosteroids have also been tried experimentally in the prevention or treatment of proliferative vitreoretinopathy (Tano et al. 1980, Jonas et al. 2000), retinal neovascularization (Antoszyk et al. 1993, Danis et al. 1996), and choroidal neovascularization (Challa et al. 1998, Danis te al. 2000). After injection of IVT, the drug is delivered rapidly to its site of action with maximal bioavailability. Animal studies have shown that the intravitreally injected suspension maintains a depot lasting 21 to 41 days (Schindler et al. 1982, Scholes et al. 1985). In addition, triamcinolone has vitreous half-life of 1.6 days compared with 2.5 hours for dexamethasone, a glucocorticoid (Scholes et al. 1985). Recent studies have suggested that IVT injection may be effective in reducing the DME and improving the BCVA (Jonas et al. 2001, Martidis et al. 2002, Massin et al. 2004, Chieh et al 2005). Interestingly, TA has not been found in clinically significant concentrations in serum shortly after intravitreal injections of about 20 mg TA, suggesting that major systemic side-effects may not be very probable (Degenring and Jonas 2004). It agrees with clinically observations that the metabolic control of patients with DM is not markedly influenced by the intraocular application of the steroid. TA as a treatment option for DME can be given in many forms: IVT injection, subtenon injection, and in combination with vitrectomy as an adjunctive procedure (as in our third study). Massin and associates (Massin et al. 2004) included in their comparative study 15 consecutive patients with bilateral DME unresponsive to laser photocoagulation therapy. All patients received a unilateral intravitreal injection of about 4 mg TA. They detected a significant reduction in macular thickness and slight, however not statistically significant, increase in BCVA in the injected eyes compared with the contralateral eyes without IVT injection. In this study the duration of a reduction of the macular thickness as measured by OCT was less than 6 months. At the end of the fellow-up, visual acuity measurements returned to the baseline values with no significant difference between baseline values and the measurements obtained at the end of the fellow-up. In 6 of the 12 injected eyes, intraocular pressure exceeded 25 mmHg, and was controlled by topical medication. Jonas and associates (Jonas et al. 2001) studied in a prospective study the clinical outcome and complications of TA as an adjunctive procedure in patients undergoing pars plana vitrectomy for treatment of complicated PDR. They suggested that IVT injection (15 to 20 mg) with most of the vehicle removed seems to be well tolerated by eyes undergoing vitrectomy for PDR. They documented a pseudohypopyon consisting of TA crystals in the inferior anterior chamber angle detected in one patient and resolved spontaneously within 4 days. Again Jonas and associates in more recent study (Jonas et al. 2003) reported their results about IVT injection as an additional tool in vitrectomy for PDR. They found that eyes that underwent vitrectomy with IVT injection compared with the nonrandomized controlled group without IVT injection did not show a higher than usual rate of postoperative complications. Avci and associates (Avci et al.2006) studied in a recent prospective, interventional consecutive case series study consisted of 59 eyes with chronic DME, which received a 4 mg IVT injection. All patients completed at least 6 months follow up. The mean BCVA improved significantly at the third postinjection month. However, the macular oedema reached the pretreatment level in 29 (49%) of the eyes at 6 months and 15 of 21 eyes (71%) at 9 months after injection. In the light of the previous studies, IVT injection seems to be effective in reducing the DME and improving the BCVA, however the effect might be short term and the

reinjection might be required in many cases. In our study we did not apply the TA in an injection form; we applied it at the end of vitrectomy as an adjuvant potential treatment. Although, the safety of IVT has been supported by results of prior animal studies and some human trials (McCuen et al. 1981, Hida et al. 1986, Kivilcim et al. 2000, Young et al. 2001), many side-effects and complications related to the therapy have been reported. The potential complications of IVT may be injection related or from the effect of corticosteroid suspension. One of the most common side-effects of IVT is the steroid-induced elevation of intraocular pressure (Wingate and Beaumont 1999, Martidis et al. 2002, Bakri and Beer 2003, Jonas et al. 2003, Chieh et al. 2003, Jonas et al. 2003). Diagnosis of DM or presence of CSME did not influence the reaction of intraocular pressure after the injection (Jonas et al. 2005). It may agree with previous randomized clinical trials in which DM was not a major risk factor for glaucoma (Palmberg 2001). Jonas and associates (Jonas et al. 2004) reported that IVT injection of approximately 20 mg of TA can increase intraocular pressure beyond 21 mmHg in up to 40% of patients, and that in most patients, the TA-induced rise in intraocular pressure can be treated topically, except approximately 1% of patients who must undergo filtering surgery. Previous comparing studies using different dosages of IVT injection may suggest that the higher the dosage is, the longer is the duration of secondary ocular hypertension (Wingate and Beaumont 1999, Bakri and Beer 2003, Jonas et al. 2003, Jonas et al.2004). In our third study we documented elevation of the intraocular pressure (< 35 mm Hg) in 7 eyes (21. 87%); all eyes could be treated by using local antiglaucoma medication within 1 month postoperatively. Infectious, sterile and pseudo endophthalmitis after IVT injection have been reported in many recent trials (Benz et al. 2003, Jonas et al. 2003, Sakamoto et al. 2004, Moshfeghi et al. 2004, Moshfeghi et al. 2005). In a multicenter study, Sakamoto and associates (Sakamoto et al. 2004) evaluate the incidence of acute endophthalmitis after TA assisted pars plana vitrectomy. Of total 1.886 cases only one case showed acute endophthalmitis due to Staphylococcus epidermidis (0.053%). Yamashita and associates (Yamashita et al. 2004) reported a case of weak endophthalmitis (Staphylococcus epidermidis) after TA-assisted vitrectomy. Four days after surgery, endophthalmitis associated with anterior chamber hypopyon was noticed, the patient's vision had deteriorated to hand motion. In the spite of severe cell infiltration, the ciliary injection and ocular pain were not significant. Additional vitrectomy with irrigation of antibiotics was performed, and then the endophthalmitis was soon resolved. On the other hand Jonas and Bleyl (Jonas and Bleyl 2004) reported that some eyes with endophthalmitis after IVT show a marked destruction of the whole globe. As a steroid, TA may inhibit the immigration of the inflammatory cells into those areas in which the TA crystals are present, which may be paralleled by the clinical observation that patients with infectious endophthalmitis after an IVT injection usually show almost no pain which is rather uncommon for infectious endophthalmitis in eyes without intraocular steroids (Nelson et al. 2003). Sterile endophthalmitis after IVT injection have been reported (Nelson et al. 2003, Parke 2003). It was suggested that it could be related to the solvent agent. Jonas and associates (Jonas et al. 2000) documented cases with pseudo-endophthalmitis after IVT injection. If TA crystals are washed from the vitreous cavity into the anterior chamber, they deposit down in the inferior anterior chamber angle mimicking a hypopyon, which is difficult to differentiate from the painless hypopyon caused by a post-injection infectious endophthalmitis. In our third study we documented acute bacterial endophthalmitis (Staphylococcus epidermidis) in 1/32 (3.1%) eye required additional vitrectomy with intravitreal application of antibiotics and injection of silicon oil. The high frequency of this complication in our study is certainly explained by the small number of patients included in the study. TA injected into the vitreous cavity may lead to de-arrangement of the structure of the vitreous body, which may exerts traction on the retina leading to a rhegmatogenous retinal detachment. If TA is applied at the end of

vitrectomy and rhegmatogenous retinal detachment occurred after the surgery, it is more likely that retinal detachment is related to the vitrectomy not to the TA. In our third study we documented retinal detachment in 1/32 (3.1%) required additional peripheral vitrectomy with fluid-gas (16 % C3F8) exchange. Jonas and associates (Jonas et al. 2005) reported that in elderly patients, 20 mg IVT injection leads to clinically significant cataract in about 15% to 20% of eyes within about one year after the injection. If TA injected at the end of vitrectomy, cataract formation could be related to the both procedures, IVT and the vitrectomy. In our third study we documented cataract formation in 2/32 (6.25%) eyes during the fellow-up period (mean 5.5 months). These results suggest that IVT injected at the end of vitrectomy may induce cataract formation in fewer cases than only IVT injection without vitrectomy. The rate of cataract formation in our study was small compared to Jonas's study but this might be related also to the shorter follow-up period in our study. In the light of the previous studies, IVT may have a positive effect, however may be also a transit effect in reducing DME and improving BVCA, therefore reinjection is required in some cases. Furthermore IVT injection is not risk-free procedure; therefore, in our third study we injected IVT at the end of vitrectomy to treat patients with diffuse DME, refractory to laser photocoagulation treatment. The purpose was to increase the chance of the treatment success by using the combination of vitrectomy and IVT and to avoid possible complications related to the injection it self. Vitrectomy has been advocated for the treatment of DME; however, vitrectomy may be applicable only to a specific subset of diabetics with DME and requires a significant surgical intervention with its inherent risks, recovery time, and expense. IVT seems theoretically a promising treatment for refractory diffuse DME, but further studies are required to demonstrate that it safely improves visual acuity. The occurrence of a relapse may justify retreatment, whose tolerance and frequency will also have to be evaluated. IVT injected into the eye at the end of vitrectomy may be effective in reducing DME, however includes some risks related to the corticosteroid suspension. IVT for DME is a new treatment option and long-term experience has not been available yet. For date there are many open questions about the use of IVT for DME unanswered yet.

In our histopathologic study the features of cellular elements attached to the ILM mostly resembled macrophage. Asami and associates (Asami et. al. 2004) ultrastructurally observed collagen fibers on the vitreous side of the surgically removed ILM from eyes with DME. In the ILM of the human diabetic retina, it has been already reported that the amount of other extracellular matrix components such as fibronectin (Kohno et al. 1987, Ljubimov et al.1996), laminin (Kohno et al. 1987), and Type I, III, IV and collagen (Ljubimov et al.1996) are increased. Tano (Tano 2000) has reported that ILM taken from DME cases were indeed almost twice as thick as the ILM taken from MH cases. Avci (Avci et al. 2004) did not found any differences in the thickness between ILM peeled from eyes with DME and those with MH. In our histopathologic study the thickness of the peeled ILM in the DME group and the MH group was significantly increased. Some cellular elements (mostly resembled macrophage) were observed on the vitreous side of the peeled ILM in the both groups; however, they were not seen on the retinal side. In agreement with previous studies (Asami et al. 2004, Avci et al. 2004) we conclude that ILM thickening and cell abundance on the vitreous surface might contribute to the course and the pathogenesis of DME and idiopathic MH.

8. SUMMERY AND CONCLUSIONS

Diabetic macular edema is the most common cause of visual impairment in diabetic patients. Precise pathophysiology of diabetic macular edema is unclear and seems to be multifactorial and includes pericyte loss, microaneurysm formation, basement membrane thickening and

focal closure of the capillary bed, vitreomacular traction, and ultimately breakdown of the blood-retinal barrier with increased vascular permeability. Risk factors for clinical significant diabetic macular edema are hyperglycemia, hypertension, hyperlipidemia, duration of diabetes, and pregnancy. The increasing number of individuals with diabetes worldwide suggests that diabetic macular edema will continue to be major contributors to vision loss and associated functional impairment in the working-age population of most developed countries. Although eyes with diffuse macular edema carry a particularly poor prognosis despite laser photocoagulation, laser treatment is still the first choice of treatment for diabetic macular edema as it is safety and less invasive that other surgical options. Diffuse diabetic macular edema is characterized by diffuse leakage from extensive areas of the posterior retinal capillary bed, a scarcity of hard exudates, and often the formation of cystoid spaces. Focal macular edema, in contrast, is characterized by focal leakage from microaneurysms and dilated capillary segments and is moor responsive to laser photocoagulation. Laser photocoagulation for diabetic macular edema is mainly sight preserving and not sight resorting. The failure of laser photocoagulation in a substantial subgroup of patients has prompted interest in other treatment methods, including surgical treatment with vitrectomy with or without peeling of the internal limiting membrane, and application of the intravitreal steroids. In conclusion:

1. The findings of our first study in agreement with previous studies support the effectiveness of vitrectomy with or without internal limiting membrane peeling in resolving diabetic macular edema in majority of eyes. This however, is not always associated with visual acuity improvement. Better outcomes were achieved in eyes with evident vitreomacular traction and with short duration of the edema. The discrepancy between anatomic and functional results may relate to irreversible changes of the macula due to long-standing macular edema, therefore optimal timing of the surgery seems to be important prognostic factor. We confirm that DME is good indication for vitrectomy, especially in eyes with vitreomacular traction by the adherent thickened posterior hyaloid following a partial posterior vitreous detachment. Eyes with attached posterior hyaloid in the macular region, but without thickening and without traction on the macula and eyes with detached posterior hyaloid could also benefit (but in less percentage) from vitrectomy. Vitrectomy seems to involve the multifactorial pathogenesis of diabetic macular edema.

2. The findings of our second pilot study demonstrated that in eyes with diabetic macular edema, refractory to laser treatment and without evident epimacular proliferation, vitrectomy without internal limiting membrane peeling was as effective in reducing the foveal thickness and improving the visual acuity as vitrectomy with internal limiting membrane peeling. We conclude that peeling of the internal limiting membrane is not essential for anatomic and visual success in diabetic macular edema surgery. Internal limiting membrane peeling might be indicated on a case- by- case base, not as a standard procedure during vitrectomy for diabetic macular edema. Furthermore iatrogenic macular hole or lamellar defect can be associated with ILM peeling in eyes with large cystoid spaces composed of thin inner retinal layer.

3. In our third study and in according with previous studies we found that application of triamcinolone acetonide into the vitreous cavity at the end of vitrectomy may have a positive effect in reducing the macular edema and improving the visual acuity, however the complications of triamcinolone may include serious complication as endophthalmitis. Vehicle removal and triamcinolone application must be performed under strict sterile conditions. Because of the novelty of this therapy, one has to be very careful since long-term experience has not been available yet. There are many questions unanswered yet, such as the optimal dosage, the mode of application, are there other complications than those already reported? Is

it necessary to remove the solvent agent prior to the intraocular injection and how should be removed.

4. In our fourth study and in agreement with previous studies we conclude that enhanced thickening of the internal limiting membrane and cell abundance on the its vitreous surface might contribute to the course and the pathogenesis of diabetic macular edema and idiopathic macular hole.

5. Vitrectomy and creating of posterior vitreous detachment for diabetic macular edema refractory to laser treatment might be more effective than traditional management of observation or further laser and might offer a longer lasting effect than injection of triamcinolone. These surgical results have made us considerably reduce the use of laser photocoagulation for diabetic macular edema and decide for earlier intervention in selective cases. However, one should keep in mind that vitrectomy requires a significant surgical intervention with its inherent risks, recovery time, and expense.

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