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RETINAL DISORDERS

Pars plana vitrectomy for diabetic macular edema. Internal limiting membrane delamination vs posterior hyaloid removal. A prospective randomized trial

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Abstract
Background Diabetes mellitus, as well as subsequent ocular complications such as cystoid macular edema (CME), are of fundametal socio-economic relevance. Therefore, we evaluated the influence of internal limiting membrane (ILM) removal on longterm morphological and functional outcome in patients with diabetes mellitus (DM) type 2 and chronic CME without evident vitreomacular traction.
Method Forty eyes with attached posterior hyaloid were included in this prospective trial and randomized intraoperatively. Prior focal (n=31) or panretinal (n=25) laser coagulation was permitted. Group I (n=19 patients) underwent surgical induction of posterior vitreous detachment (PVD), group II (n=20 patients) PVD and removal of the ILM. Eleven patients with detached posterior hyaloid (group III) were not randomized, and ILM removal was performed. One eye had to be excluded from further analysis. Examinations included ETDRS best-corrected visual acuity (BCVA), fluorescein angiography (FLA) and OCT at baseline, 3 and 6 months postoperatively. Main outcome measure was BCVA at 6 months, secondary was foveal thickness.
Results Mean BCVA over 6 months remained unchanged in 85% of patients of group II, and decreased in 53% of patients of group I. Results were not statistically significant

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different [group I: mean decrease log MAR 95% CI (0.06; 0.32), group II: (−0.02; 0.11)]. OCT revealed a significantly greater reduction of foveal thickness following PVD with ILM removal [group I: mean change: 95% CI (−208.95 μm; −78.05 μm), group II: (−80.90 μm: +59.17 μm)].
Conclusion Vitrectomy, PVD with or without ILM removal does not improve vision in patients with DM type 2 and cystoid diabetic macular edema without evident vitreoretinal traction. ILM delamination shows improved morphological results, and appears to be beneficial in eyes with preexisting PVD.

Keywords Vitrectomy · Posterior vitreous detachment · Internal limiting membrane · Optical coherence tomography · Cystoid diabetic macular edema

Introduction

In former investigations the vitreous and the vitreoretinal junction have been detected as a modulator of diabetic macular edema [1–7], and their precise role in the pathogenesis of diabetic maculopathy is widely debated [8]. Encouraged by reports showing that diabetic patients with posterior vitreous detachment (PVD) are less likely to develop macular edema [6] and that resorption may occur after a spontaneous PVD [2, 9], vitrectomy with removal of the posterior hyaloid was considered to be a therapeutic option in diffuse and cystoid edema, which often persists despite multiple laser treatments [5, 7, 10]. Lewis reported successful vitrectomy in selected patients with diabetic macular edema associated with visible posterior hyaloidal traction [5]. Furthermore, clinical improvement has been reported in eyes even when macular traction was stated to be absent [4, 11–15]. Tachi and Ogino reported on a prospective series of 58 eyes from 41 patients with visual acuity improvement in 53% of cases. However, they performed a combined cataract extraction in most of them [13]. Several authors postulated a positive effect of additional peeling of the internal limiting membrane [ILM] in patients with diabetic macular edema refractory to laser photocoagulation [1, 16–18]. Most reliable data on the efficacy of vitrectomy and PVD was published by Otani and Kishi and Stolba and coworkers, first reported on a non-randomized series of seven patients after unilateral vitrectomy with PVD and significant reduction of macular edema in comparison with the fellow eye [12]. Stolba et al. recently demonstrated in a prospective randomized trial that vitrectomy with ILM removal provides better visual and morphological results than the natural course [19]. Uncertainty remains as to whether vitrectomy and PVD alone is sufficient, or whether additional ILM delamination is beneficial. A prospective non-randomized study by Patel et al. indicated that ILM removal achieves an improved morphological outcome [20]. Gandorfer et al. showed a remarkable decrease of macular edema, with improvement of BCVA [1]. However, in most studies a reduction of macular edema did not consequently lead to a better visual acuity [3, 11].

Aim of this prospective randomized study was to evaluate the efficacy of surgical PVD and additional ILM removal in comparison to vitrectomy and PVD alone in typical diabetes mellitus type 2 patients with cystoid diabetic macular edema (CME) and without evident vitreomacular traction.

Due to the ILM removal, human material became available and was used to study sequestered factors, which could be involved in the pathogenesis of early diabetic retinopathy [21]. A further aim of the study was therefore to look for factors in the ILM possibly inducing vascular permeability changes.

Methods

Demographic data

The prospective randomized study population consisted of 50 patients (22 female, 28 male; 19 right eyes, 32 left eyes). Pars plana vitrectomies were performed at the Department of Ophthalmology, University Clinics Schleswig-Holstein, Campus Luebeck for cystoid diabetic macular edema between July 1999 and June 2003. Patients were divided into three groups: group I: surgical PVD, group II: surgical PVD and ILM removal, group III: preexisting PVD, vitrectomy and ILM removal. One eye of group I was excluded from further analyses because both eyes of one single patient had been randomized. Thirty eyes presented non-proliferative diabetic retinopathy (NPDRP) (group I: 73.68%; group II: 60%, group III: 36.36%) and 20 eyes proliferative diabetic retinopathy (PDRP) (group I: 26.32%; group II: 40%; group III: 63.64%). Nine out of these revealed active but mild neovascularizations of the disc or elsewhere; the others were quiescent. No fibrovascular tissue or traction was present. None of the patients showed a taut thickening of the posterior vitreous hyaloid by biomicroscopy, neither preoperatively, nor intraoperatively. Hard exudates in the macular region were present in 38 patients (76%). None of the patients revealed an enlarged foveolar avascular zone on fluorescein angiography (FA). A detachment of the neurosensory retina from the retinal pigment epithelium or any sign of epiretinal membrane or vitreoretinal traction was ruled out by biomicroscopy and by optical coherence tomography (OCT).

At baseline, systemic hypertension controlled with medications was present in 12/19 patients in group I, 15/

20 patients in group II and 11/11 patients in group III. Insulin-dependent diabetes mellitus was present in 13/19 patients in group I, 15/20 patients in group II and 9/11 patients in group III. Mean HbA_{1c} levels were 7.59 in group I, 8.15 in group II and 7.85 in group III. The demographic data for each group are listed in Table 1.

Informed written consent was obtained for all patients, and the study protocol was approved by the local ethics committee.

Study protocol

The eligibility criteria for this study included cystoid macular edema unresponsive to one or more laser treatments (interval at least 3 months since last photocoagulation). Only patients with diabetes mellitus type 2 were enrolled in the study. Eyes with evident vitreomacular traction, macular non-perfusion spreading over an area of 2 disc diameters, optic atrophy, subfoveal plaques or vitreous hemorrhage were excluded, as well as eyes with dense cataract or other ocular diseases. Vitreomacular traction or epiretinal membranes were excluded by OCT. The main outcome measure was BCVA determined by ETDRS charts after 6 months. Secondary endpoint was foveal thickness measured by OCT after 6 months.

Intraoperatively, after a preexisting PVD was ruled out, the patient was either randomized to group I (surgical PVD only) or group II (surgical PVD and ILM removal). In all of them, a detachment of the posterior hyaloid was surgically induced. An additional ILM delamination was performed in group II. In 11 patients, the posterior hyaloid was intraoperatively found not to be attached to the retina, as had been presumed preoperatively. These eyes (22%) were not randomized, but underwent ILM removal, and were followed by the regular examination regimen to evaluate the effect of ILM removal alone in patients with cystoid diabetic macular edema and preexisting PVD. They were summarized in group III. The participant flow chart according to the CONSORT statement [22] is shown in Fig. 1.

Examinations

Preoperatively and at 3 and 6 months postoperatively, all eyes underwent ETDRS visual acuity testing, a careful biomicroscopic evaluation, measurement of the foveal area thickness using optical coherence tomography (OCT II and III, Zeiss–Humphrey, Jena, Germany) and fluorescein angiography. All regular examinations were performed by the same examiner (MM). The visual acuity was evaluated following the guidelines of the study protocol of the "Early Treatment Diabetic Retinopathy Study Research Group" (ETDRS) [23]. The postoperative visual outcomes of the treatment groups were compared at 3 and 6 months.

Macular edema at baseline and during the follow-up was assessed by fluorescein angiography in terms of size and

Table 1 Demographic data and patients characteristics at baseline in the treatment groups

Characteristics	Group I (PVD)	Group II (PVD+ILM-peeling)	Group III (ILM-peeling)
Number of patients	19	20	11
Gender (*n*): male/female	10/9	12/8	6/5
Mean age (years)	66.0 (SD 8.96)	63.55 (SD 7.14)	70.0 (SD 6.23)
Preop. visual acuity (logMAR, mean)	0.59 (SD 0.23)	0.59 (SD 0.23)	0.65 (SD 0.27)
Preoperative foveal thickness (μm, mean)	425.25 (SD 83.25)	442.13 (SD 83.73)	478.9 (SD 134.86)
Lens-status (*n*):			
- phakic	17	19	8
- pseudophakic	2	1	3
Type of diabetic retinopathy (*n*):			
- non-proliferative	14 (73.63%)	12 (60%)	4 (36.36%)
- proliferative	5 (26.32%)	8 (40%)	7 (63.64%)
Prior laser treatment (*n*):			
- panretinal	7	9	9
- focal/grid	16	15	10
Hypertension (*n*)	12	15	11
HbA_{1c} level (%, mean)	7.59 (SD 1.17)	8.15 (SD 1.60)	7.85 (SD 1.24)
Serum creatine level (μmol/l, mean)	78.33 (SD 28.10)	78.00 (SD 20.84)	108.00 (SD 41.15)
Mean duration of diabetes mellitus (years, mean)	14.75 (SD 6.56)	12.67 (SD 5.40)	16.56 (SD 9.55)
Mean duration of macular edema (months, mean)	10.40 (SD 8.32)	10.29 (SD 7.14)	22.22 (SD 20.20)
Insulin dependent/non-insulin dependent (*n*)	13/6	15/5	9/2

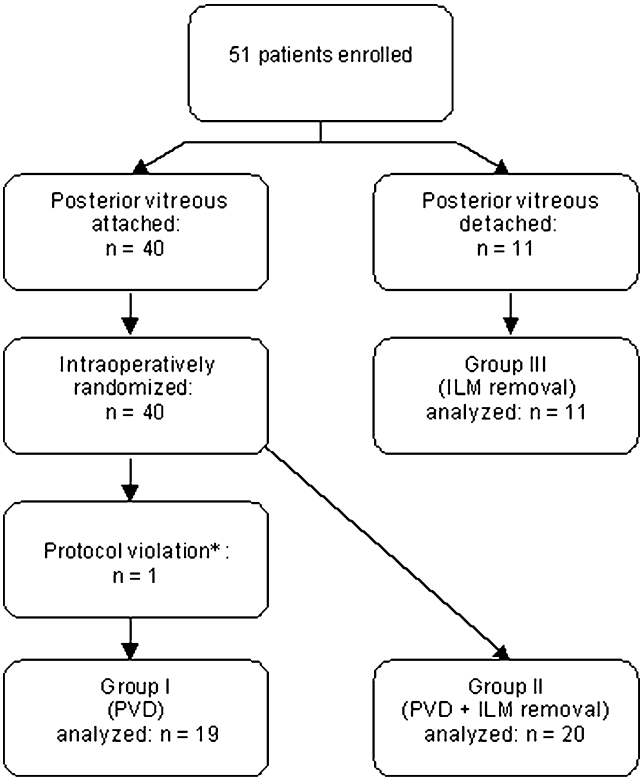

Fig. 1 Participant flow chart for each treatment group. *PVD*= posterior vitreous detachment, *ILM*=internal limiting membrane, * two eyes of one single patient were randomized

intensity, and by OCT measurements to determine the foveal thickness, which was defined as the distance between the inner retinal surface and the retinal pigment epithelium at the central fovea. The fovea was scanned in a vertical direction.

To analyze the long-term outcome within this prospective study and to minimize the influence of cataract formation, we decided to perform a late follow-up examination of the patients, including BCVA and foveal thickness in addition to the study protocol. Long-term visual acuity values were obtained in part at our institution (ETDRS-VA) and in part by the referring ophthalmologists (Snellen VA). Longterm OCT thickness measurements could be obtained only in patients who were re-examined in our institution. Results are analyzed non-confirmatively.

Surgical procedure

After written informed consent was obtained, all operations were performed by a single surgeon (HH) under general anaesthesia. A three-port pars plana vitrectomy was performed, and following core vitrectomy the posterior vitreous cortex was detached from the retina by suction over the optic nerve disc until the Weiss ring was identified. No triamcinolone was used for this manoeuver, in order to exclude a possible influence on the visual or morphological outcome.

In patients randomized to group II and in patients of group III, the ILM was removed using endgripping forceps. The first 12 patients (nine of group II and three patients of group III) underwent ILM removal without the use of dyes; in the subsequent 19 patients (eleven of group II and eight patients of group III) the ILM was stained with approximately 0.1 ml of indocyanine green (ICG-Pulsion, Munich, Germany) dissolved in 5% glucose in a concentration of 0.05% under constant irrigation with Ringer's solution. Immediately after application, dye remnants within the vitreous cavity or outside the central retina were washed out by suction using a flute needle or the ocutome. The ILM removal was extended over the whole edematous area to the vascular arcades, and was performed successfully in all eyes. The removed tissue was confirmed to be ILM by light microscopy, and in part further examined by immunohistochemistry. Intraoperatively, panretinal argon endolaser coagulation was performed in 11 of the 50 eyes (group I: three eyes, group II: five eyes, group III: three eyes) for the treatment of severe non-proliferative (n=2) or proliferative diabetic retinopathy (n=9). No focal or grid laser therapy was applied intraoperatively or within the follow-up period. Then, a careful examination of the retinal periphery by scleral indentation was carried out. Cryocoagulation was performed for peripheral iatrogenic tears (n=10; group I: three eyes, group II: five eyes, group III: two eyes). No endotamponade was intended, but was necessary for peripheral tears in ten eyes (gas: n=4, air: n=6).

Statistical analysis

Statistical analysis was performed using SAS (the SAS System; Release 9.1.3 SP 2; SAS Institute Inc., Cary, NC, USA) on Windows® 2000 SP 4 (Microsoft Corp., Redmond, WA, USA). Values are given as frequency and percentage for qualitative, and mean and standard deviation for quantitative parameters respectively. 95% confidence intervals were calculated using SAS PROC MEANS. Analyses of variance (with repeated measures for time: preoperatively and at 3 and 6 months) were performed to evaluate time and treatment effects on visual acuity (logMAR) and foveal thickness (OCT).

Immunhistochemistry of surgically excised ILM

Surgically removed ILM specimens of 24 patients were examined by immunhistochemistry. Twelve specimens were from patients of group II from our study, and 12 from idiopathic macular holes without diabetes mellitus served as control. The specimen were fixed in 4% buffered formalin and embedded in paraffin. Eight-micrometer (8 μm)-thick paraffin sections were cut and mounted onto silanized

slides. ABC staining technique (Vector Laboratories/Linaris, PK-6200, Wertheim-Bettingen, Germany) was used. Slides were blocked in normal horse serum for 30 minutes at room temperature (RT). Primary antibodies were mouse monoclonal bFGF (Calbiochem, GF22, 1:100, Darmstadt, Germany), CML (Novo Nordisk, 2F8, 1:1000, Malmö, Sweden), methylglyoxal advanced glycation endproduct (AGE) antibody (Dr. M. Brownlee, Albert Einstein Institute of Medicine, New York, NY, USA, 1:500), tumor necrosis factor (TNF) alpha (Calbiochem, CN 654300, 1:100, Darmstadt, Germany), VEGF (Calbiochem, GF-25, 1:20, Darmstadt, Germany), platelet-derived growth factor (PDGF)-B (Santa Cruz, sc-7878, 1:500, Heidelberg, Germany), and a goat polyclonal interleucin (IL)-6 (Santa Cruz, sc-1266, 1:100, Heidelberg, Germany). The slides were incubated for 1 hour at room temperature. After three washing steps for 5 minutes in PBS, the sections were incubated with the biotinylated secondary antibody (ABC Kit) for 1 hour at room temperature, followed by a second washing step. DAB as substrate was used for detection. Photos were taken by Leica digital camera and IM 50 software.

Results

Visual acuity

Group I After vitrectomy, mean BCVA in group I decreased from 20/80 (logMAR mean 0.59, SD 0.23) preoperatively to 20/100 (logMAR mean 0.70, SD 0.28) at 3 months and 20/125 (logMAR mean 0.78, SD 0.34) at 6 months. BCVA improved more than 2 lines in one of 19 eyes (5.3%), remained the same in nine eyes (47.4%) and worsened in nine eyes (47.4%).

Group II After vitrectomy, PVD and additional ILM delamination, mean BCVA (preoperatively 20/80 (logMAR mean 0.59, SD 0.23)) decreased to 20/100 (logMAR mean 0.65, SD 0.28) at 3 months and 20/100 (logMAR mean 0.64, SD 0.21) at 6 months. BCVA improved more than 2 lines in one of 20 eyes (5%), remained the same in 17 eyes (85%) and decreased in two eyes (10%).Comparing groups I and II, the functional outcome did not reveal a significant treatment effect, taking into account the repeated measures ($p=0.5492$), but did show a significant time effect ($p=0.0040$): at 6 months, group I: mean decrease 0.19 logMAR, SD 0.27, 95% CI [0.06; 0.32], group II: mean decrease 0.05 logMAR, SD 0.14, 95% CI [−0.02; 0.11]).

Group III In group III, patients having preexisting PVD, the mean preoperative BCVA was 20/100 (logMAR mean 0.65, SD 0.27). The mean postoperative BCVA after vitrectomy and ILM removal was unchanged at 20/100 (logMAR mean 0.68, SD 0.28) at 3 months, and increased to 20/80 (logMAR mean 0.60, SD 0.29) at 6 months postoperatively. BCVA improved more than 2 lines in three of 11 eyes (27.3%), remained the same in six eyes (54.5%) and worsened in two eyes (18.2%). Since this group was not randomized, no confirmative comparison with the other groups was performed.

The functional results are presented in Fig. 2.

Foveal thickness measurement

Group I In patients of group I, mean preoperative foveal thickness was 425.25 μm (SD 83.25 μm). Three months postoperatively, the mean foveal thickness in group I was 432.33 μm (SD 77.26 μm), and at 3 months 415.2 μm (SD 132.25 μm). There was no significant effect of time ($p=0.9198$).

Group II In patients of group II, mean preoperative foveal thickness was 442.13 μm (SD 83.73 μm). Three months postoperatively, the foveal thickness in group II decreased to a mean of 352.62 μm (SD 102.38 μm). Six months postoperatively, it

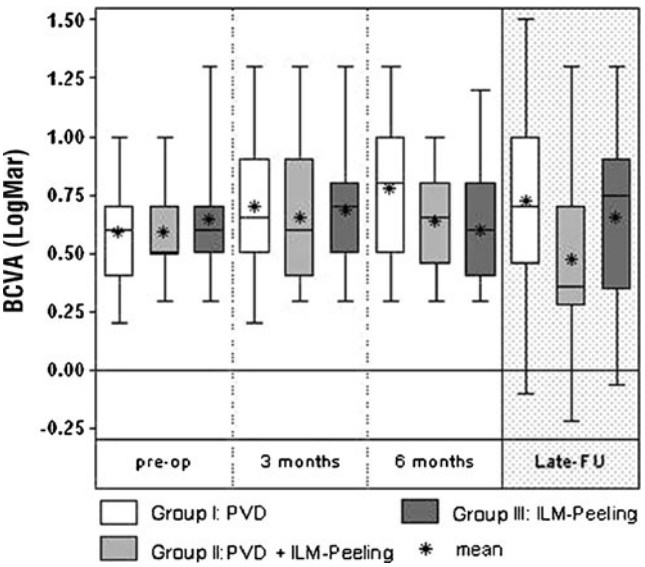

Fig. 2 Functional results of all groups, showing a decrease of best-corrected visual acuity (BCVA) in group I with PVD only and stable BCVA in groups II and III with additional ILM removal. The influence of cataract progression has to be considered within the 6-month follow-up period. Late follow-up BCVA values could be obtained only in part at our institution. *PVD*=posterior vitreous detachment, *ILM*=internal limiting membrane

further decreased to a mean of 297.64 μm (SD 139.92). This group showed a significant effect of time (p=0.0034). Comparing groups I and II, no significant treatment effect was detected (p= 0.0871). However, in group II with ILM removal, an increased resolution of edema was found at 3 months [mean difference to preoperative value −93.92 μm, SD 122.28 μm, 95% CI: (−167.81; −20.03) compared to a mean difference of −7.42 μm, SD 62.43 μm; 95% CI: (−47.08; 32.25) in group I] and at 6 months [mean difference −143.50 μm, SD 113.36 μm, 95% CI: (−208.95; −78.05) compared to −10.87 μm, SD 126.47 μm, 95% CI: (−80.90; 59.17) in group I].

Group III In group III patients having preexisting PVD, the preoperative mean foveal thickness was 478.90 μm (SD 134.86 μm). Three months after vitrectomy and ILM removal the foveal thickness decreased to a mean of 308.13 μm (SD 121.20 μm), and after 6 months to 296.60 μm (SD 121.42 μm). As this group has not been randomized, no comparison with other groups was performed.

The anatomic results of all groups measured by OCT are presented in Fig 3.

Late follow-up-examination

Group I At a mean late follow-up of 56.01 months (SD 10.0 months) the functional data of 17 patients were available. Despite cataract extraction that had been performed in 13/16 phakic patients, mean BCVA remained unchanged at 20/100 (logMAR mean 0.72, SD 0.45), but mean foveal thickness of eight patients at 54.01 months (SD 8.0 months) further decreased to 357.0 μm (SD 95.09 μm).

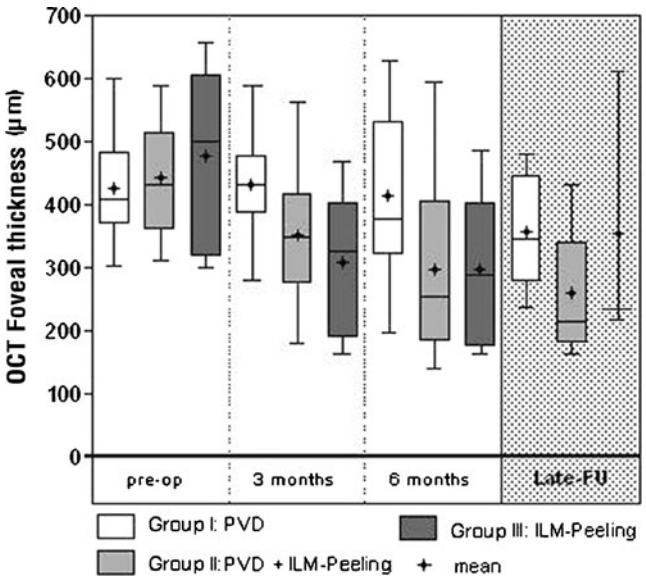

Fig. 3 Mean foveal thickness of treatment groups over the follow-up period, showing only a slight decrease of edema in group I and markedly better reabsorption in groups II and III with additional ILM removal. Late follow-up OCT values could be obtained only in some of the patients

Group II At a mean late follow-up of 46.0 months (SD 17.0 months), cataract extraction had been performed in 13/19 phakic patients. BCVA of 17 patients at that time had decreased again to 20/63 (logMAR mean 0.47, SD 0.3). Correspondingly, mean foveal thickness of 12 patients measured at 49.0 months (SD 5.0 months) had slightly increased to 258.0 μm (SD 96.50). Comparing the randomized treatment groups I and II at their late follow-up visit, the functional outcome in group II was favourable [group I: logMAR mean 0.16, SD 0.39, 95% CI: (−0.05; 0.36) group II: logMAR mean −0,09, SD 0,32, 95% CI: (−0.25; 0.08)], but it has to be considered that only 17 patients of each of groups I and II were analyzed and in part the visual acuity values were obtained by the referring ophthalmologists (Snellen VA). The morphological outcome at the late follow-up visit again was favourable in group II: [group I: mean difference −65.43 μm, SD 124.63 μm, 95% CI: (−180.69;49.83), group II: −184.11 μm, SD 141.78 μm, 95% CI: (−293.09;−75.13)], but only in eight patients of group I and in 12 patients of group II an OCT measurement could be obtained. The mean decrease of foveal thickness in group II was 37.37% at the late visit [SD 24.82, 95% CI: (18.29, 56.45)], whereas in group I it was 13.53% at the late visit [SD 27.18, 95% CI: (−11.61; 38.67)].

Group III At the late follow-up visit at 48.0 months (SD 16.00) postoperatively, cataract extraction had been performed in all phakic patients (n=8). BCVA of eight patients at that time was 20/100 (logMAR mean 0.65, SD 0.43). The mean foveal thickness measured only in three patients at 49.0 months (SD 8.00) was 354.67 μm (SD 223.04 μm; single values: 235.0 μm, 217.0 μm, 612.0 μm). The mean decrease was 28.92% [SD22.45, 95% CI: (−26.84, 84.68)].

Subgroup analysis — indocyanine green

The first nine patients of group II and three patients of group III underwent ILM removal without the use of dyes. In 19 consecutive patients (11 of group II and eight of group III), an ICG-assisted peeling of the ILM was

performed. Mean BCVA in the ICG group decreased slightly from 20/80 [logMAR 0.59, SD 0.19, 95% CI: (0.5; 0.68)] preoperatively to 20/80 [logMAR 0.61,SD 0.21, 95% CI: (0.5; 0.71)] at 6 months and remained stable at the follow-up. Mean BCVA in the group without the use of ICG decreased from 20/80 [logMAR 0.64, SD 0.19, 95% CI: (0.44; 0.84)] preoperatively to 20/100 [logMAR 0.65, SD 0.28, 95% CI: (0.47; 0.83)] at 6 months, and increased to 20/50 [logMAR 0.36, SD 0.34, 95% CI: (0.1; 0.63)] at the late follow-up visit. Comparing both groups, ICG did not influence the functional outcome.

With regard to the morphological outcome, patients with the use of ICG revealed a decrease of mean foveal thickness from 483.94 μm (SD 112.4 μm, 95% CI: (442.02; 543.85)] preoperatively to 347 μm [SD 116.85 μm, 95% CI: (284.74; 409.26)] at 3 months, to 347.06 μm [SD 128.51 μm, 95% CI: (278.58; 415.54)] at 6 months and to 325 μm [SD 161.02 μm, 95% CI: (176.08; 473.92)] at the late follow-up visit. In patients without the use of ICG the mean foveal thickness preoperatively was 408.67 μm [SD 77.21 μm, 95% CI: (349.31; 468.02)], at 3 months 299.4 μm [SD 80.35 μm, 95% CI: (199.63; 399.17)], 197.5 μm [SD 53.65 μm, 95% CI: (152.65; 242.35)] at 6 months and 235.63 μm [SD 74.6 μm, 95% CI: (173.26; 297.99)] at the late follow-up visit.

Hard exudates

Group I The numbers of hard exudates increased in 7/15 patients (46.66%), remained the same in 3/15 patients (20%) and decreased in 5/15 patients (33.33%) at the 6-month visit in patients who underwent posterior vitreous detachment only.

Group II The number of hard exudates increased in 6/18 patients (33%), remained the same in 2/18 patients (11%) and decreased in 10/18 patients (56%) 6 months postoperatively when an additional ILM delamination was performed.

Group III In group III with preexisting PVD, the number of hard exudates increased in 1/6 patients (16.5%), remained the same in 1/6 patients (16.5%) and decreased in 4/6 patients (67%) 6 months after vitrectomy and ILM removal.ILM removal in groups II and III was qualitatively found to increase resorption rate of hard exudates.

Fluorescein angiography

Group I The extension of the edema enlarged in 5/19 patients (26.32%), remained unchanged in 6/19 patients (31.58%) and diminished in 8/19 patients (42.11%) at 6 months. The intensity at that time increased in 7/19 patients (36.84%), remained unchanged in 1/19 patients (5.26%) and reduced in 11/19 patients (57.89%). The foveolar avascular zone (FAZ) had slightly enlarged in six patients and decreased in one patient.

Group II The extension of the edema enlarged in 2/20 patients (10%), remained unchanged in 8/20 patients (40%) and decreased in 10/20 patients (50%) at 6 months. The intensity at that time increased in 3/20 patients (15%), remained unchanged in 3/20 patients (15%) and reduced in 14/20 patients (70%). In two patients the FAZ slightly enlarged and diminished in three patients. Comparing groups I and II, additional ILM delamination did not qualitatively influence intensity or extension of edema.

Group III In group III with preexisting PVD, the extension of edema enlarged in 1/11 patients (9%), remained unchanged in 5/11 patients (45.5%) and decreased in 5/11 patients (45.5%) at 6 months. The intensity at that time increased in 2/11 patients (18%), remained unchanged in 2/11 patients (18%) and decreased in 7/11 patients (64%). In one patient the FAZ slightly enlarged, and in one patient FAZ decreased.

Complications

Iatrogenic tears were observed intraoperatively in ten patients (n=3 in group I, n=5 in group II, n=2 in group III) and treated by cryocoagulation (n=10) and air (n=6) or gas endotamponade (n=4).

Intraoperative iatrogenic opening or rupture of macular cysts was not observed in any patient. In 42/44 (95.45%) phakic patients, cataract formation with nuclear sclerosis was observed within 6 months (n=15 in group I, n=19 in group II, n=8 in group III).

Epiretinal membrane formation occured in one patient of group I and in one patient of group II. One patient of group II required re-vitrectomy and silicone oil tamponade for a dense postoperative vitreous hemorrhage. In three patients, a retinal detachment developed (group I: n=1, group II: n=1, group III: n=1). These patients underwent successful revitrectomy with encircling band (n=1), radial sponge (n=2), and gas endotamponade (n=1).

Immunohistochemical results

ILMs of patients with diabetic maculopathy intensely stained for bFGF and methylglyoxal-type AGEs (Fig. 4).

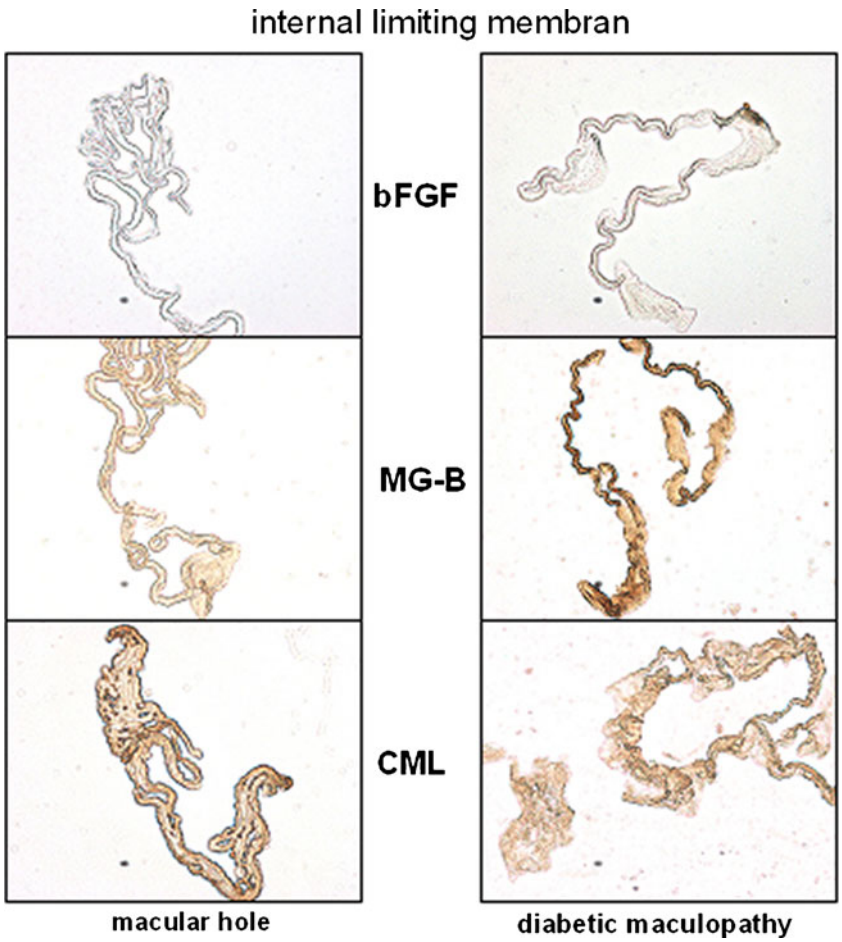

Fig. 4 Histologic section of a surgically removed internal limiting membrane from a patient of group II showing immunostainings for bFGF, methylglyoxal-type AGE and CML. Note that in comparison with ILM from patients with macular holes, there is no difference in CML deposition, but a substantial difference for methylglyoxal-type AGE, and, to a minor degree bFGF

In contrast, the glycoxidation marker CML was not different when compared with ILMs from patients with idiopathic macular holes. ILMs stained negatively for VEGF, PDGF-B, TNFalpha and IL-6.

Discussion

Few data from randomized controlled trials are available concerning the therapeutic effect of vitrectomy for diabetic macular edema. Recently, Stolba et al. reported a favourable outcome following vitrectomy and ILM peeling opposed to the natural course [19]. Yanyali et al. also observed a decrease in retinal thickness, without, however, significant improvement of visual acuity [15]. Uncertainty exists as to the necessity of ILM removal or whether PVD alone is sufficient. In a retrospective study, Stefaniotou et al. found that ILM peeling was beneficial in patients with diffuse diabetic macular edema [24]. In contrast, Bahadir and colleagues could not prove a beneficial effect of vitrectomy and ILM removal in comparison to vitrectomy alone [25]. No significant improvement of functional results in patients with diffuse diabetic macular edema was observed by Kumar et al. comparing vitrectomy with removal of ILM in contrast to grid laser photocoagulation [26].

The presented study provides the first randomized controlled trial data demonstrating a favourable effect of additional ILM removal in vitrectomy for cystoid diabetic macular edema in diabetes type 2 patients without evident vitreomacular traction, as shown in foveal thickness measurements by OCT. In the long term, while PVD alone slowly improves the anatomical results, it is markedly less effective than additional ILM removal. Although, the morphological results were substantial, visual results were unsatisfactory. Comparing the results of groups I and II, it can be stated that ILM removal shows the tendency of being beneficial in eyes with primarily attached vitreous in which a PVD was surgically induced analyzing the secondary endpoint of foveal thickness. However, identification of the posterior hyaloid by slit-lamp contact biomicroscopy is notoriously difficult, and even OCT can visualize the vitreoretinal interface only when it is partially detached or where the PVD is located within a few micrometers from the retinal surface. This was the reason why the randomization was performed intraoperatively, after a preexisting vitreous detachment was definitively

ruled out by the surgeon. In group I, the surgeon relied only on the visualization of the Weiss ring during the PVD induction, based on careful observation and surgical experience. No triamcinolone was used for the identification of vitreous remnants, in order to exclude any influence of drug remnants on the resorption of the macular edema. An incorrect intraoperative judgement of the posterior vitreous may have influenced the results. Even during surgery, it may be hard to distinguish a PVD from vitreoschisis, especially in patients with diabetic retinopathy. In those 20 eyes where ILM removal was performed, it is extremely unlikely that the posterior vitreous was still attached, especially in patients in whom ICG was used for ILM removal, since dyes such as ICG facilitate visualization of vitreous remnants.

Moreover, our results suggest that even when a spontaneous PVD is present (group III), vitrectomy with ILM removal may provide favourable morphological results. It has to be emphasized that this group III was not randomized, and that the number of included patients in all groups was small. Due to this limiting drawback of the present study, subgroup evaluation was complex, and the conclusions must be looked at with caution. Nevertheless, the study design was prospective.

The most distinct effect of our study seen on fluorescein angiography was not in the decrease in edema size but rather the reduction of staining intensity. This parameter is difficult to quantify and OCT has become an important tool, providing detailed morphologic information and allowing the detection of macular edema with a sensitivity of 89% and a specificity of 96% [1, 27, 28]. However, the resolution available in OCT systems is not high enough to distinguish between intra- and extracellular edema.

The exact pathomechanism of ILM removal on diabetic macular edema is currently unknown. The ILM, which is the basement membrane of the Müller cells, may act as a diffusion barrier. Recent findings of a comparative histopathological study of surgically removed ILM showed that the ILMs in patients with diabetic macular edema were significantly thicker (mean thickness: 4.8±1.6 μm), revealing a larger amount of cellular elements on the vitreous side than in patients with macular holes (1.8±0.6 μm) [1, 29]. This thickened ILM may act as a barrier, decreasing transretinal fluid movement. After removal of this diffusion barrier, the disturbance of transretinal fluid movement may be in part restored and contribute to a resolution of the macular edema [1]. Also, Radetzky et al. speculated about a pseudomembrane formed by the endplates of Müller cells exerting a barrier function [30]. Intraretinal structural damage in chronic cystoid edema may further influence the diffusion properties.

It is also possible that the thickened ILM may reduce the diffusion of oxygen from the vitreous cavity to the retina following vitrectomy. Stefánsson et al. have suggested that fluid currents in the vitreous cavity following vitrectomy of PVD transport oxygen from well-perfused areas of the retina to hypoxic areas such as in diabetic macular edema [31, 32]. The diffusion of oxygen from the fluid in the vitreous cavity into the retina would be retarded by a thickened ILM.

Another theory is that the repair mechanisms of Müller cells may be activated by delamination of the ILM [33]. Also, the absence of the vitreous gel would increase the transport of cytokines, such as VEGF, from the retina into the vitreous cavity, and the absence of ILM would further speed up this clearance of cytokines from the retina [8].

The efficacy of ILM delamination may be caused by the removal of a growth factor reservoir which may have accumulated in the ILM and in cellular elements on its vitreous side. A recent study showed that vitreous remnants may be present after surgical vitreous separation [34]. Histopathologic correlations by Gandorfer et al. [35] and Matsunaga et al. [29] support the theory that ILM delamination allows a more complete removal of vitreous elements.

As Fig. 4 demonstrates, CML and AGEs could be detected within removed ILM specimens of patients with diabetic macular edema of groups II and III, in contrast to those gained during macular hole surgery. AGEs have been implicated in the primary pathogenesis of diabetic vascular damage that ultimately leads to progressive vascular occlusions, as in diabetic maculopathy. Furthermore, AGEs can enhance VEGF production and thereby induce permeability changes. The finding of bFGF in the ILMs of diabetic eyes supports previous data showing bFGF deposits in the inner limiting membranes of retinae from diabetic patients [36], and of experimentally diabetic animals [37]. It is worth noting that bFGF is an important repair/survival factor for retinal glia suggesting that our finding is consistent with increased repair, rather than a sign of damage. The glycoxidation product CML is present in a variety of conditions associated with increased oxidative or glycative stress, in particular in the eye [38–40]. The finding that methylglyoxal-type AGE is found in membranes representing the endfeet of Müller cells is of particular clinical interest, given the present debate concerning the role of neuroglia in the course and pathogenesis of diabetic retinopathy, and the possible role of these specific AGEs in the link between metabolic stress and transcriptional regulation of genes in Müller cells [41]. AGEs can enhance VEGF production, and thereby may induce permeability changes.

Compared to the promising anatomical findings, the functional results in our study are less beneficial. The additional ILM delamination still seemed to stabilize visual acuity with an unchanged or better BCVA in 90% of patients, despite development of a marked complicating cataract in most patients within the follow-up period. There was a statistically significant effect over time, revealing higher

visual deterioration of BCVA in group I without ILM removal [mean decrease of 0.19 logMAR, SD 0.27, 95% CI (0.06; 0.32 logMAR)] than in group II [mean decrease 0.05 logMAR, SD 0.14, 95% CI (−0.02;0.11)] but without treatment difference. To rule out the influence of cataract formation at the end of the study, we decided to additionally determine long-term visual and morphological results. This extension of the study was not planned in the initial study protocol. Most of the patients had undergone cataract extraction in the meantime, but surprisingly there was no remarkable change among the groups. Only group II showed improved VA results compared to baseline; groups I and III remained stable.

Our limited functional results in group I stand in contrast to recently published studies about vitrectomy without ILM peeling in diabetic macular edema. La Heij et al. reported on the resolution of diabetic macular edema in 21 of 21 eyes [11], and Tachi and Ogino in 57 of 58 eyes [13]. However, both studies based their findings on fluorescein angiography and biomicroscopy, which makes an objective measurement of retinal thickness difficult if not impossible. Furthermore, the study results of La Heij are completely based on follow-up examinations from referring ophthalmologists only, which raises doubt about the standardization of measurement conditions. Ikeda et al. performed vitrectomy on three eyes of two patients in which evidence of macular traction was specifically noted to be absent. They observed resolution of macular edema with improvement of visual acuity in all eyes [4]. In a recent retrospective study, Rosenblatt et al. also reported significant improvement of visual acuity and diminution of retinal thickness in patients with refractory diabetic macular edema without taut posterior hyaloid [18]. The preexisting compromised retinal microvasculature in diabetic eyes is likely to be more vulnerable to exsudation when subjected to traction. Therefore, it is understandable that vitrectomy and posterior vitreous separation was beneficial in selected patients with diabetic macular edema when the hyaloid has been judged to be taut [5, 7, 10]. However, the patients in our study represent the typical situation in most elderly diabetic mellitus type 2 patients where no clinically visible vitreo-macular traction is present. Although there has been a considerable number of studies implicating an important role of the posterior hyaloid in the pathogenesis of diabetic macular edema [11–13, 16], we did not find any effect of posterior hyaloid detachment alone in our study.

One possible cause for the discrepancy of those studies in comparison with our results is the longer history of macular edema resulting in cystoid changes in a high number of patients, and the selection of diabetic mellitus type 2 patients in our patient series, which may have had a negative impact on photoreceptor recovery in this elderly study population. The presence of cystoid macular edema in our study population is a clinical sign for the chronic nature of the edema and longstanding intraretinal diabetic changes with irreversible photoreceptor damage. Moreover, the limited functional results in our study may have been influenced by bad systemic glucose control (HbA_{1c}: 7.59–8.15, see Table 1) [42].

Futhermore, visual outcome might have been adversely affected by one or more prior grid laser photocoagulations, treatment which was an inclusion criterion for our study. During surgery in 11 patients, panretinal argon endolaser coagulation due to severe non-proliferative or proliferative diabetic retinopathy was performed, in ten patients peripheral cryocoagulation and endotamponade for peripheral iatrogenic tears. These findings had no statistical consequence on functional or anatomic outcomes. However, as group sizes are fairly small, we cannot rule out an additional impact.

Dillinger and Mester also observed a discrepancy between the anatomical and functional outcome; however, they still reported a significant visual improvement of 43%, gaining 2 lines of vision or more. In accordance with our positive results in groups II and III, the authors reported a decrease in macular thickening in 55 of 60 eyes after vitrectomy and ILM removal in chronic diabetic macular edema [16]. Our study was performed prospectively, but the quality of follow-up was limited due to postoperative examinations by the referring ophthalmologists and loss of standardized conditions.

Recently, in a prospective non-randomized study, Patel et al. confirmed the beneficial effect of ILM removal on morphological outcome, but also found limited visual improvement [20]. They suspected that the normal Müller cell physiology may be altered after ILM removal, which may explain the lack of significant improvement in vision despite a marked resolution of macular edema. We could not show a significant effect of ILM removal; however, a tendency towards improved results after PVD with additional ILM removal could be seen.

Since ICG should allow a more complete ILM removal, better morphological functional results could be expected in the subgroup of patients in which it was used for staining. However, in our study ICG seemed to affect visual and morphological outcome negatively. Avci et al. reported about spontaneous ILM detachment from the neurosensory retina after ICG-assisted ILM peeling in patients with diabetic macular edema, using 0.25 % ICG solution [43] for 1 minute after total fluid–air exchange. We used a markedly lower concentration of 0.05 % dissolved in glucose under constant irrigation. But the observed tendency to poor results using ICG corresponds with other reports on possible toxicity of ICG. Brilliant Peel® was not available at that time.

The complication spectrum and rate is comparable to other macular surgery studies, with a higher rate of retinal tears and retinal detachments after surgically induced posterior vitreous detachment.

In conclusion, our study underlines the efficacy of PVD with additional ILM removal on resorption of

cystoid diabetic macular edema, even without the presence of vitreomacular traction. Improvement or at least stabilization most likely can only be achieved when the macular edema is reduced. A further visual deterioration such as in group I is very likely if the macular edema persists. A negative influence of ILM removal on the visual outcome could not be proven by our data. The procedure seemed to stabilize visual acuity, and therefore this treatment option might be considered in earlier stages of the disease to preserve vision at higher levels. However, it has to be kept in mind that the study design did not allow the assessment of the efficacy of vitrectomy in diabetic macular edema against the natural course or grid laser coagulation, which is the current gold standard. Whether it is preferable to perform this procedure as a first-line treatment for diffuse diabetic macular edema instead of grid laser coagulation must be evaluated in another trial. New treatment modalities such as intravitreal triamcinolone acetonide [44–46] or anti-VEGF drugs [47, 48] are promising, but recurrences and the requirement of multiple re-injections are the major backdraw of presently available drugs. As our late analysis suggests, vitrectomy and ILM removal is able to stabilize visual and morphological results also on the longterm.

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