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Triple therapy: Phaco-vitrectomy with ILM peeling, retinal endophotocoagulation, and intraoperative use of Bevacizumab for diffuse diabetic macular edema

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: The aim of this study was to evaluate the visual acuity and structural outcomes of combined phaco-vitrectomy with ILM peeling, retinal endophotocoagulation and use of bevacizumab in patients with diffuse diabetic macular edema (DDME).

Material/Methods: In this prospective, nonrandomized, interventional study we included 29 eyes of 26 patients with DDME. The best-corrected visual acuity (BCVA) and central retinal thickness and volume (CRT and CRV) were recorded at 4, 8, 12, and 16 months after surgery.

Results: The mean preoperative BCVA was 0.74±0.36 logMAR (0.3–1.5) and improved finally to 0.4±0.24 logMAR (–0.1–1.0) p=0.000006. The mean preoperative CRT in the 1mm zone was 516±184 μm (256–950) and decreased postoperatively at the last control to 237±75 μm (117–489) p=0.000003. The mean preoperative CRV in the 1mm zone was 0.39±0.14 μL (0.19–0.74) and decreased postoperatively at the last control to 0.17±0.06 μL (0.09–0.36) p=0.000003. The mean preoperative CRT in the 6 mm zone was 407±105 μm (279–640) and decreased postoperatively at the last control to 282±40 μm (212–380) p=0.000004. The mean preoperative CRV in the 6 mm zone was 11.4±2.9 μL (7.85–17.93) and decreased postoperatively at the last control to 7.92±1.0 μL (5.62–10.97) p=0.000003. The 23 (79.3%) eyes showed improvement in BCVA ≥0.2 logMAR, 5 (17.2%) eyes improvement or stabilization of BCVA and 1(3.5%) eye deterioration. Preoperative BCVA was a positive factor for prognosis of BCVA at 12th month follow-up (b=0.42; p=0.006), while the negative factors were: previous panretinal photocoagulation (b=–0.24; p=0.04), presence of vitreomacular traction (b=–0.29; p=0.02) and preoperative CRT in the 1000 μm zone (b=–0.24; p=0.07). A greater visual acuity improvement occurred in eyes with worse baseline visual acuity (b=–1.01; p=0.00001). The presentation of vitreomacular traction (b=–0.38; p=0.02), previous panretinal photocoagulation (b=–0.31, p=0.04) and greater preoperative CRT in the 1000 μm zone (b=–0.31; p=0.07) were negative factors for visual improvement.

Conclusions: This combined treatment resulted in improvement or stabilization of BCVA and decrease of CRT and CRV. Larger comparative studies are necessary to establish the real impact of this therapeutic approach.

key words: **diffuse diabetic macular edema (DDME) • vitrectomy • ILM peeling • anti-VEGF injection • endophotocoagulation**

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Background

Macular edema is the main vision-limiting factor in diabetes and is a disorder of increasing public health importance throughout the world [1]. Diffuse macular edema results from a generalized breakdown of the blood-retinal barrier with intra- and subretinal accumulation of fluid. Based on observations of the Early Treatment of Diabetic Retinopathy Study Group, laser photocoagulation can decrease the rate of moderate visual loss up to 50%; however, visual improvement is uncommon (3–14.5%) in DME [2]. Moreover, outcomes with laser treatment in diffuse diabetic macular edema are more unfavorable and some eyes have persistent edema and visual loss. This fact has led investigators to seek alternative modalities for the management of DDME, such as administration of steroids, protein kinase C (PKC) inhibitors or vascular endothelial growth factor (VEGF) inhibitors by intravitreal injections. The pharmacotherapeutic approach is under investigation to determine if certain drugs, either alone or in combination with focal/grid laser, result in superior visual acuity outcomes compared with laser alone.

VEGF is involved in pathogenesis of diabetic macular edema [3,4] and recently anti-VEGF agents such as bevacizumab and ranibizumab have been shown to be beneficial in the treatment of this retinal disorder [5–8]; however, endogenous VEGF is required for visual function. A growing body evidence indicates that VEGF also acts on nonvascular cells; it plays a survival role in Müller cells and photoreceptors [9]. Therefore, anti-VEGF therapies should be administered with caution and not in a permanent manner. Photocoagulation in nonperfused areas eliminates increased production of VEGF, induces proliferation of RPE and increases production of PEDF in the surrounded impact laser area [10].

Posterior hyaloids [11–13] play an important role in the development of diffuse macular edema. The observation that the release of mechanical traction on the macula with subsequent reduction in DME, either by spontaneous posterior vitreous detachment or with vitrectomy, lends support to this line of reasoning [14–16]. A proper understanding of the anomalous posterior vitreous detachment (APVD) is essential for the development of an efficient strategy in treatment of diabetic maculopathy. It is possible that subclinical vitreoretinal traction on the macula exists in a large number of individuals with diabetes and may exert subclinical but significant traction on the compromised diabetic macular vascular bed and cause macular edema. The natural course of the disease leads to the fusion of intraretinal spaces in the macula and formation of a partial-thickness (Figure 1), and finally a full-thickness, extensive macular hole (Figure 2). Fibrovascular proliferations over the retinal surface can lead to retinoschisis of the macula, which, due to the lack of microvascular scaffold, is especially susceptible to tractions. (Figure 3).

It is important for the surgeon to determine the factors that might influence surgical outcome so that patients who can benefit most are chosen for the procedure. We therefore evaluated the possible preoperative and intraoperative factors that might influence surgical outcomes.

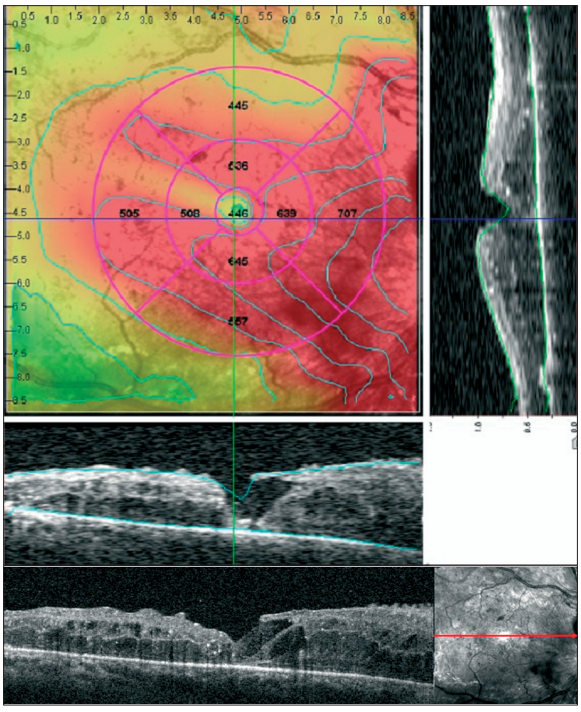

Figure 1. Partial-thickness macular hole formations during long-lasting diabetic macular edema.

Material and Methods

A prospective nonrandomized study was conducted at the Department of Ophthalmology Military Institute of Medicine in Warsaw (Poland) between September 2008 and September 2010. The study included 29 eyes of 26 patients with DDME that had recurrent or persistent macular edema after laser photocoagulation. The study was conducted under bioethical committee permission, as injection of bevacizumab into the vitreous cavity is an off-label use. All patients provided written informed consent.

The research project has been positively assessed and registered with Clinical Trials (ClinicalTrials.gov Identifier: NCT01218750).

Inclusion criteria were

1. Diagnosis of DDME upon clinical exam, definite retinal thickening involving the center of the macula, confirmed by fluorescein angiography, with or without PVD;
2. BCVA of 0.3 or worse in logMAR units (≤70 ETDRS letter) and 1.5 or better in logMAR units (≥10 ETRDS letter);
3. Mean central macular thickness greater than 250 μm on optic coherence tomography (OCT);
4. Presence of vitreomacular traction, or a thickened and taut posterior hyaloids, or presence of an epimacular membrane.

Exclusion criteria were

1. Significant macular ischemia defined as enlarged perifoveal capillary loss (>3 mm^2) by fluorescein angiography;

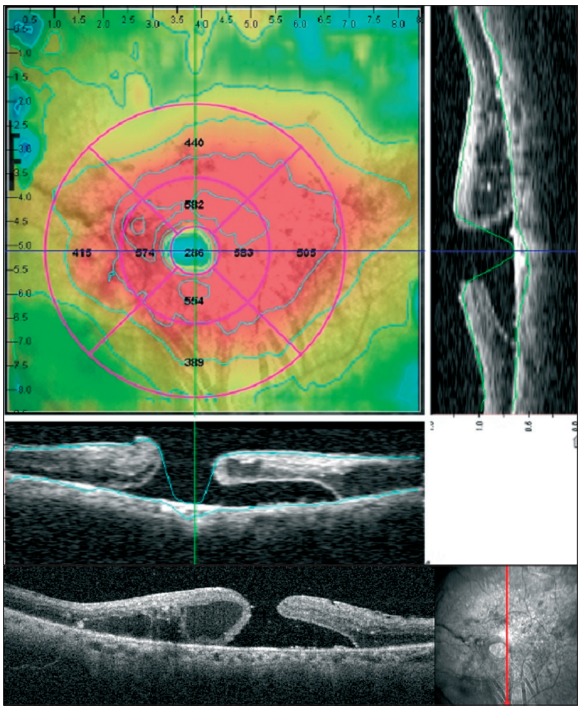

Figure 2. Extensive, full-thickness macular hole with epiretinal membrane and diffuse macular edema.

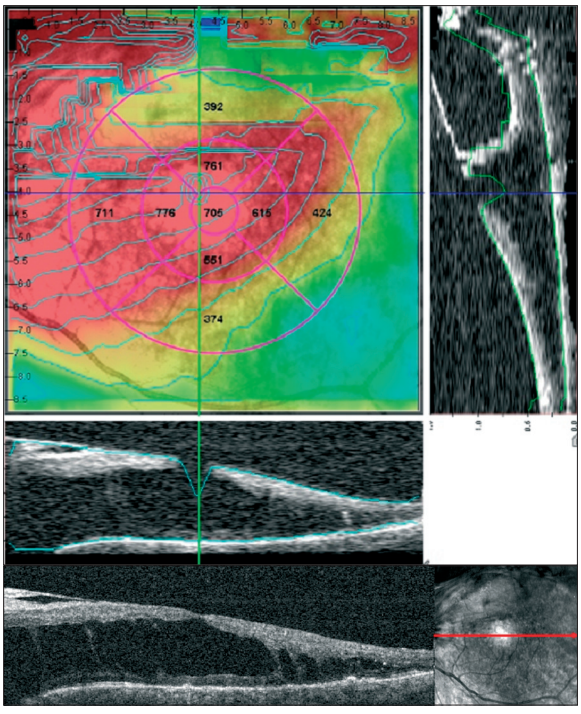

Figure 3. Fibrovascular proliferations on the optic nerve head and through tempotal superior arcades with tractional retionschisis on the macula.

2. Focal macular edema due to focal leakage from microaneurysm;
3. Ophthalmic disorders associated with macular edema, such as uveitis, branch or central retinal vein occlusion and pseudophakic cystoid macular edema;
4. Vitreous hemorrhage or tractional retinal detachment secondary to diabetic retinopathy;
5. An ocular condition is present such that visual acuity would not improve from resolution of macular edema (eg, foveal atrophy, pigmentary abnormalities, dense subfoveal hard exudates);
6. History of retinal macular photocoagulation, intravitreal corticosteroids, or other treatment for DME within the 3 months prior to enrollment;
7. History of any intraocular surgery within the prior 6 months.

The examination included the following elements: the best corrected visual acuity (BCVA) for ETDRS chart, measurement of intraocular pressure, slit lamp biomicroscopy with iris examination and lens status by LOCS, retinopathy stage by stereo examination of the posterior pole with dilatation of the pupil, history of laser photocoagulation, fluorescein angiography with evaluation of the foveal avascular zone area, and SLO-OCT were assessed preoperatively and during the follow-up period. OCT was performed 1 mm and 6 mm diameter topography centered at the patient fixation point. We evaluated mean central retinal thickness (CRT) and central retinal volume (CRV) 1 mm and 6 mm in diameter, loss of the hyper-reflective line between outer/inner segments of the photoreceptors, and presence of serous macular detachment. The preoperative and postoperative BCVA were converted to logMAR for statistical analysis. A clinically significant improvement in visual acuity was defined as 2 or more improved lines of BCVA (≥10 letters). A clinically significant reduction of the central retinal thickness was defined as a reduction of at least 10% of thickness of the central retina. Diagnosis of PVD was made by OCT and supported intraoperatively if there were no vitreous cortex remnants left at the posterior pole that could have been separated from the macula and optic nerve head by suction with the vitrectomy probe.

Table 1. Characteristics of study eyes.

Grender, M: F	11: 15
Age	59±13.8 range: 27–78
HbA1c	8.03 range: 6.9–9.4
BMI	28.45 range: 20.44–41.0
Hypertension no. of patients	24
Stage of DR, NPDR: PDR	16: 13
Preoperative laser treatment FML: PRP	10: 19
Previous cataract surgery IOL: fakic	8: 21

HbA1c – glycated hemoglobin A1c; BMI – body mass index; DR – diabetic retinopathy; NPDR – nonproliferative diabetic retinopathy; PDR – proliferative diabetic retinopathy; PRP – panretinal photocoagulation; FML – focal macular laser.

The demographic characteristics of the patients including age, sex, metabolic condition, HbA1c level, body mass index, presence of systemic hypertension and ocular status, diabetic retinopathy stage, and previous laser are shown in Table 1.

Three-port pars plana vitrectomy was performed in all patients by 1 surgeon (JR). Induction of PVD, if not present, was initiated by active suction with the vitrectomy probe over the ONH and continued peripherally (Figure 4). Triamcinolone

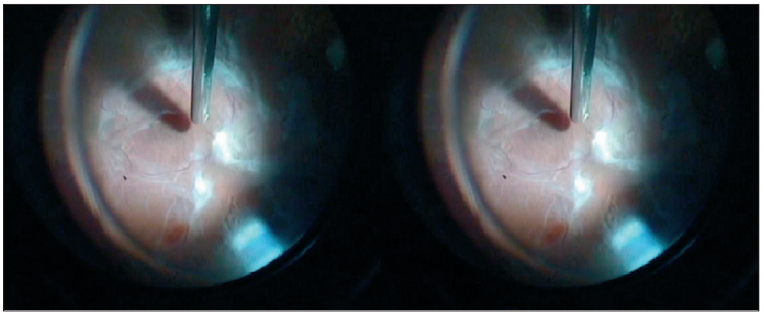

Figure 4. Posterior vitreous detachment induction by active suction with the vitrectomy probe.

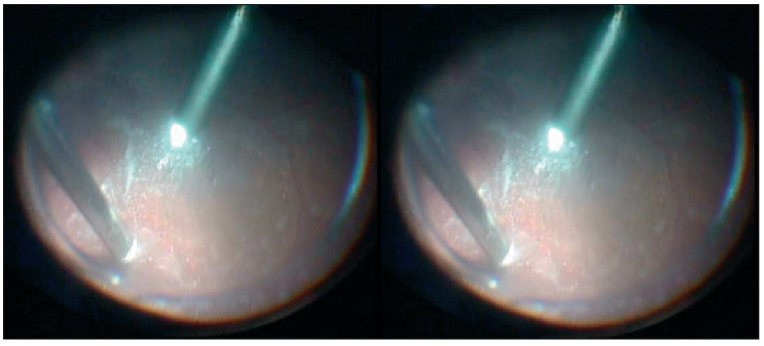

Figure 5. Triamcinolone usage for better visualization remnants of the hyaloids and epiretinal membranes on the posterior pole.

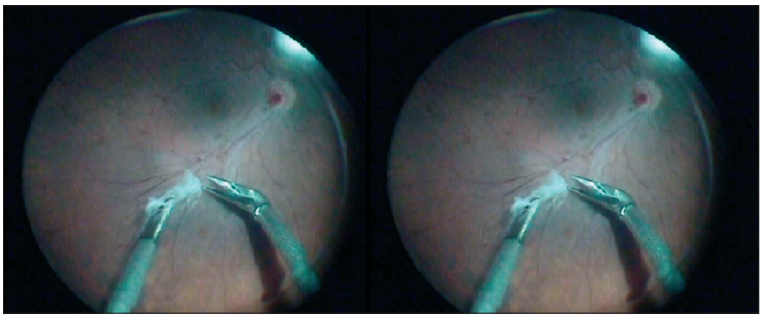

Figure 6. The removal of the epiretinal membranes by bimanual technique with an Eckardt forceps and horizontal knife.

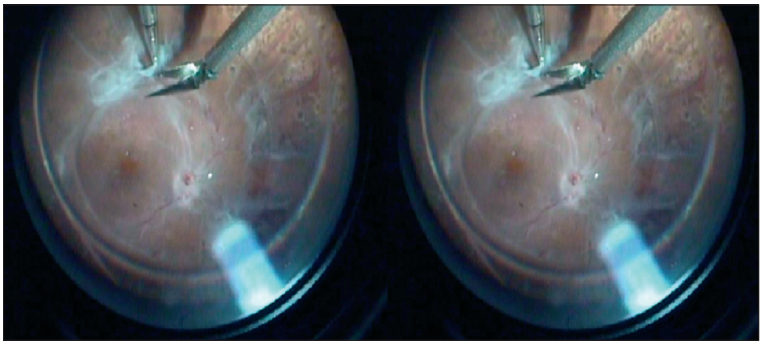

Figure 7. In difficult cases of the incipient macular detachment, stabilization of the retina in the posterior pole with perfluorocarbone was performed.

was used in all the patients only as a marker in order to visualize and removal of vitreous schisis (Figure 5). Triamcinolone was fully washed out of the eyeball during the surgery. Peeling of the epimacular tissue and ILM was performed by grasping the flap of the ILM with an Eckardt forceps (Figure 6). In difficult cases of the incipient macular detachment, stabilization of the retina in the posterior pole with perfluorocarbon was performed (Figure 7). Trypan Blue was used to stain the ILM. Peripheral laser photocoagulation was performed in cases of avascular areas based on FA, active neovascularization, peripheral retinoschisis or retinal breaks. Shaving of the peripheral vitreous body on the basis and sites of sclerotimies was very important on succesfull the surgery (Figure 8). Then a exchange fluid/air was performed by infusion and passive aspiration with the softcaniule (Figure 9). All eyes had 1.25 mg/0.05 ml of bevacizumab injected into the vitreous cavity (Figure 10) and a SF6 gas tamponade at the end of the procedure. Even in the absence of a cataract formation, a combined procedure was performed because of exact peripheral vitreous sheaving and prevention of cataract formation.

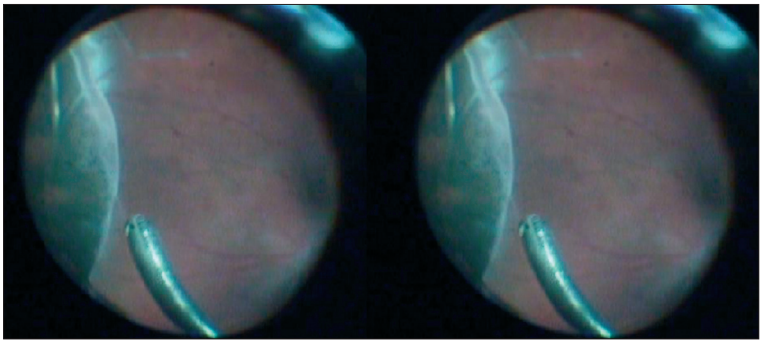

Figure 8. Shaving of the peripheral vitreous body on the basis and sites of sclerotimies.

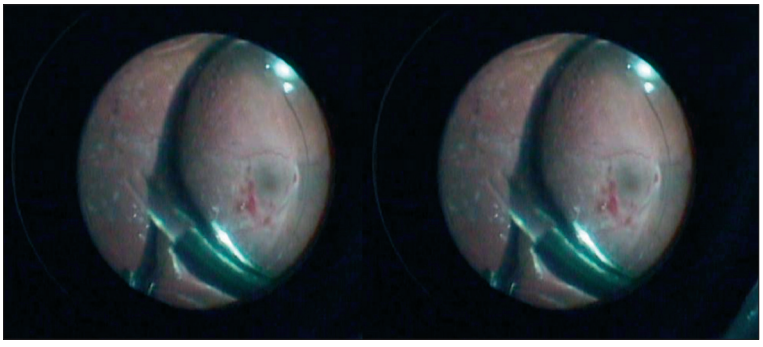

Figure 9. Exchange fluid /air by infusion and passive aspiration with the softcaniule.

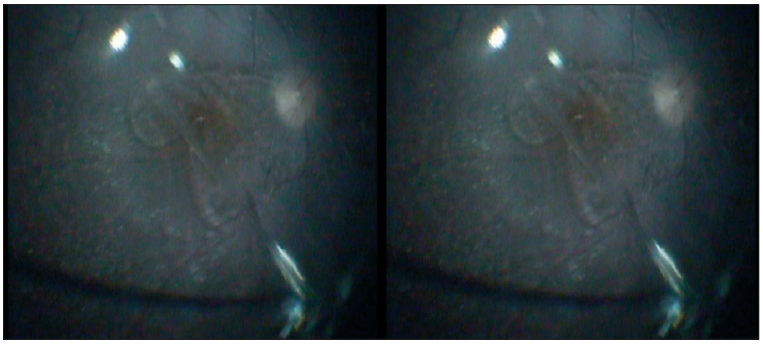

Figure 10. Delivery 1.25 mg/50 µl of bevacizumab into the vitreous cavity at the end of the surgery.

During follow-up, bevacizumab injection into the vitreous cavity and macular laser photocoagulation were conducted sequentially in case of macular edema recurrence. The injections were performer using sterile technique, 3.5 mm posterior from limbus in the inferotemporal quadrant. In the refractory cases with intraretinal cyst formation, the 25-gauge vitrectomy with drainage of the fluid from the cyst using MVB and silicone soft-tip cannula was performed.

Macular laser photocoagulation was performed directly to leaking microaneurysms in areas of retinal thickening between 500 and 3000 microns from the center of the macula and applied to all areas with edema not associated with microaneurysms. We used green diode laser VISULAS with wavelength of 532 nm, burn size 100 µm, burn duration 0.05 to 0.1 sec and enough intensity for barely visible impact.

Statistical analysis

The ANOVA rank Friedman test was used for comparison of pre- and postoperative visual acuities and central retinal thickness and volume. Multiple regression analysis was performed to evaluate the possible relationship of preoperative BCVA, preoperative central macular thickness and volume, preservation of os/is line, presentation of serous macular detachment, enlargement of FAZ, lens status, diabetic retinopathy stage, and history of laser photocoagulation with a change in postoperative BCVA, CRT in the 1000 µm zone at 12th month follow-up, visual and structural improvement.

Results

Before surgery, 19 (65.5%) eyes had previous panretinal photocoagulation, 10 eyes (34.5%) had previous macular laser photocoagulation, 7 eyes (24.1%%) had vitreomacular traction, 15 eyes (51.7%) had presence of epirenal membrane, 5 eyes (17.2%) had serous macular detachment and 2 eyes (6.9%%) had macular hole.

The mean (range) preoperative best corrected visual acuity (BCVA) was 0.74±0.36 logMAR (0.3–1.5) and improved significantly to 0.48±0.29 logMAR (0.04–1.1), p=0.000003, 0.43±0.28 logMAR (–0.02–0.9), p<0.000001, 0.42±0.29

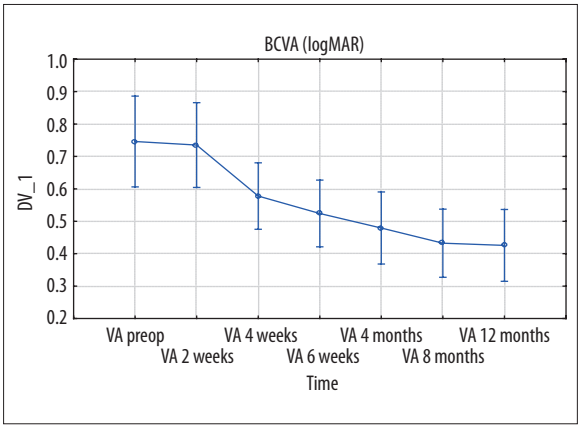

Figure 11. Pre- and postoperative BCVA. Graph demonstrating the changes in the mean best corrected visual acuity (BCVA) 2, 4, 6 weeks, and 4, 8, 12 months after triple therapy for diffuse diabetic macular edema (DDME).

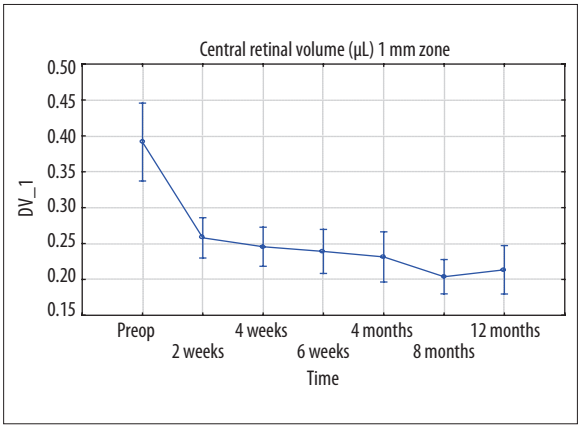

Figure 13. Pre- and postoperative central macular volume at the 1 mm zone. Graph demonstrating the changes in the mean central retinal volume (CRV) 2, 4, 6 weeks, and 4, 8, 12 months after triple therapy for the treatment of DDME.

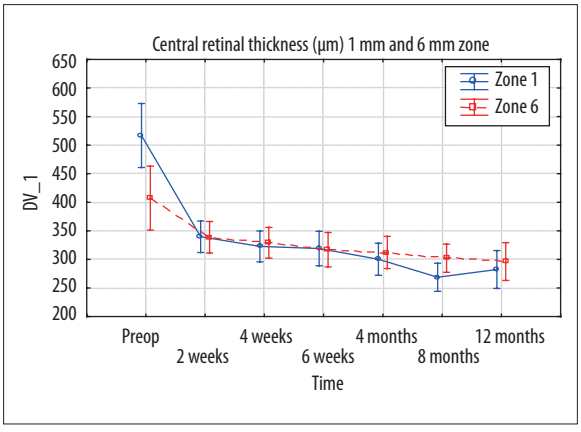

Figure 12. Pre- and postoperative central average macular thickness at the 1 mm and 6 mm zone. Graph demonstrating the changes in the mean central average retinal thickness (CRT) at the 1 mm and 6 mm zone 2, 4, 6 weeks, and 4, 8, 12 months after triple therapy for the treatment of DDME.

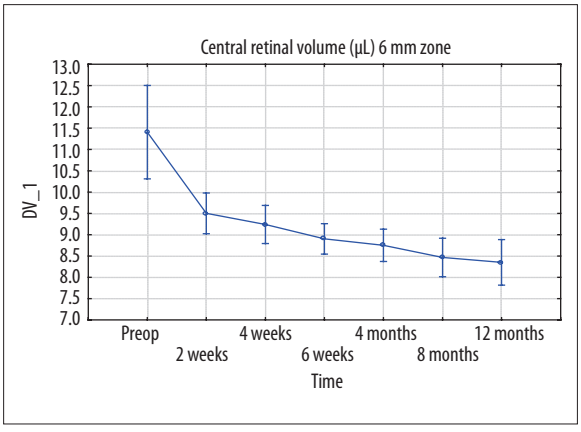

Figure 14. Pre- and postoperative central macular volume at the 6 mm zone. Graph demonstrating the changes in the mean central retinal volume (CRV) 2, 4, 6 weeks, and 4, 8, 12 months after triple therapy for the treatment of DDME.

logMAR (0–0.7), p≤0.000001 4, 8, and 12 months after triple therapy, respectively (Figure 11). The improvement of visual acuity ≥2 lines (10 letters) was in 19/29 (65.5%) eyes, 21/29 eyes (72.4%), 19/29 eyes (65.5%), 18/22 eyes (81.8%), 10/13 eyes (81.9%) 4, 8, 12, 16 and 20 months, respectively. The average improvement in visual acuity 4 months after surgery was at 13–14 ETDRS letters, 8 and 12 months at 15–16 ETDRS letters, and 16 months at 18 ETDRS letters.

The mean (range) preoperative central macular average thickness (CRT) in the 1 mm zone was 516±184 µm (256–950) and significantly decreased to 300±100 µm (115–742) p=0.000029, 268±83 µm (109–453), p=0.000004, 282±115 µm (176–606), p=0.000014 4, 8, and 12 months postoperatively. The mean preoperative central macular average thickness (CRT) in the 6 mm zone was 407±105 µm (279–640) and significantly decreased to 312±35 µm (254–407), p=0.000034, 302±41 µm (227–410), p=0.000009, 296±49 µm (272–368), p=0.000012 4, 8, and 12 months postoperatively (Figure 12).

The mean (range) preoperative central macular volume (CRV) in the 1mm zone was 0.39±0.14 µL (0.19–0.74) and decreased significantly decreased to 0.23±0.08 µL (0.09–0.55), p=0.00006, 0.2 ±0.06 µL (0.08–0.3), p=0.000005, 0.21±0.08 µL (0.08–0.46), p=0.00015 4, 8, and 12 months postoperatively (Figure 13). The mean (range) preoperative central macular volume (CRV) in the 6 mm zone was 11.4±2.88 µL (7.85–17.93) and significantly decreased to 8.75±1.0 µL (7.13–11.45), p=0.000023, 8.46±1.18 µL (6.38–11–59), p=0.000005, 8.35±1.4 µL (6.36–13.01), p=0.000007 4, 8, and 12 months postoperatively (Figure 14).

A statistically significant structural improvement occurred in all periods of observation. A clinically significant reduction, defined as a reduction of at least 10% of thickness of the central retina in the 1mm zone, occurred on the 4th and 8th month in 23 out of 29 eyes (79.3%), after 12 months in 22 out of 29 eyes (75.9%), after 16 months in 19 out of 22 eyes (86.4%), and after 20 months in 11 out of 13 eyes (84.6%).

A reduction of at least 30% of the thickness of the central retina in the 1mm zone occurred on the 4th and 12th month

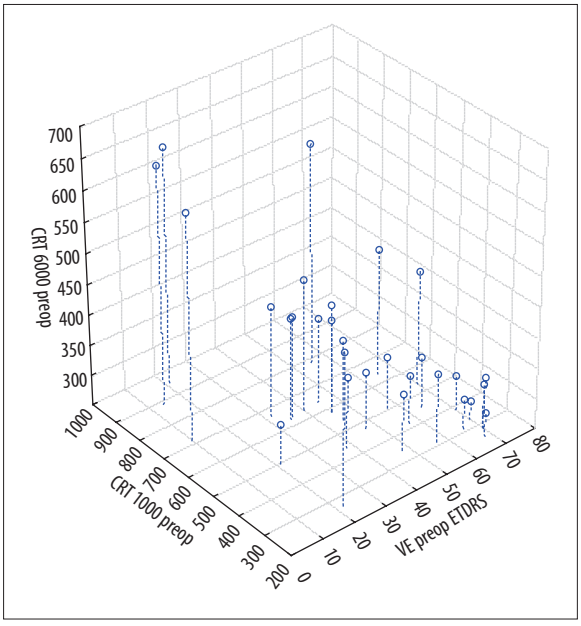

Figure 15. Scattergraph 3D for preoperative BCVA vs preoperative CRT 1000 µm *vs.* preoperative CRT 6000 µm.

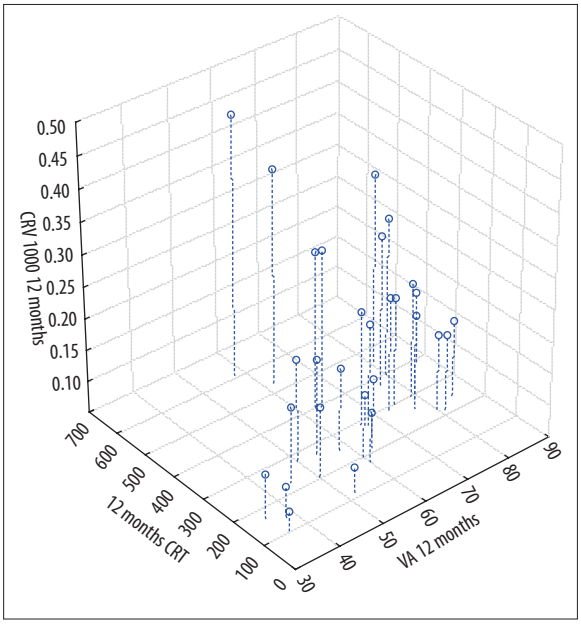

Figure 17. Scattergraph 3D for BCVA vs CRT 1000 µm *vs.* CRV 1000 µm at 12th month after triple therapy for the treatment of DDME.

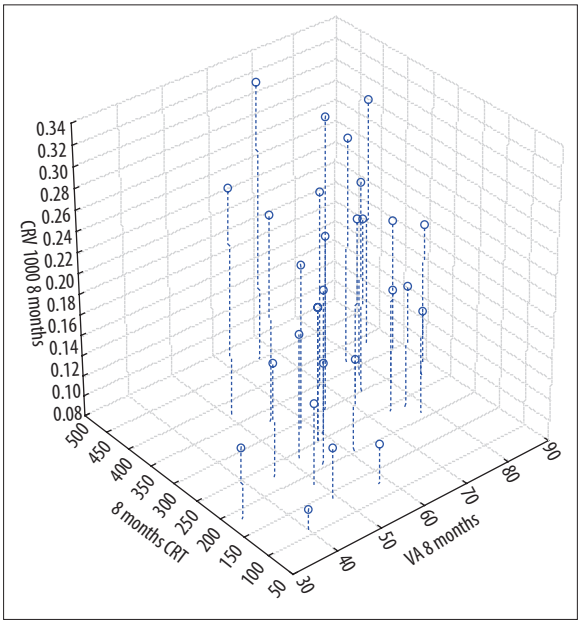

Figure 16. Scattergraph 3D for BCVA *vs.* CRT 1000 µm *vs.* CRV 1000 µm at 8th month after triple therapy for the treatment of DDME.

in 16 out of 29 eyes (55.2%), after 8 months in 18 out of 29 eyes (62%), after 16 months in 14 out of 22 eyes (63.6%), and after 20 months in 6 out of 13 eyes (46.1%).

Preoperative best-corrected visual acuity (BCVA) was negatively correlated with the average central retinal thickness and volume in the 6000 µm zone (r=–0.65, r=–0.63, p<0.0001). There was also a negative correlation with these structural parameters of the retina in the 1 mm zone (r=–0.59, p=0.001) (Figure 15).

In the 4th month of observation, the BCVA did not express statistically significant correlation with the CRT and CRV in both of the zones.

In the 8th month of observation the best BVCA had a positive correlation with the average CRT and CRV only in the 1 mm zone (r=0.54, p=0.003 and r=0.55, p=0.002). No correlation was found with the 6 mm zone (Figure 16).

In the 12th month of observation the BCVA was characterized by a worse than in the 8th month positive correlation with the CRT and CRV in the 1 mm zone (r=0.38, p=0.04 and r=0.4. p=0.033) (Figure 17).

In the 16th month of observation the 22 controlled eyes showed no correlation of the BCVA with average central retinal thickness or with average central retinal volume in both of the zones.

The anatomical relations of the central retinal characteristic of the eyes included in the study showed that the preoperative CRT was characterized by a strong positive correlation with the CRV in both the 1mm and 6 mm zones (r=0.99, p<0.0001).

During the postoperative period a sustained strong positive correlation between the central retinal thickness and volume in both the 1 mm and 6mm zones was observed (r=0.95–0.99, p<0.001 and r=0.97–0.99, p<0.001).

During the follow-up period, deterioration (0.24 logMAR) of BCVA was noted only in 1 eye (3.4%). The cause for deterioration was vitreous hemorrhage and secondary glaucoma. Two eyes (6.89%) had mild vitreous hemorrhage which resolved spontaneously at 2–4 weeks, 1 eye (3.4%) had serous macular detachment, and 2 eyes (6.89%) had posterior capsule opacifications. No eye had complications such as iris neovascularization, retinal detachments, or endophthalmitis during the follow-up period.

Table 2. Multiple regression analysis evaluating the relationship of preservation of os/is line, presentation of serous macular detachment (SMD), preoperative central retinal thickness (CRT 6000) and presentation of vitreomacular tractiom (VMT) with a chance in preoperative BCVA.

N=29	Preoperative BCVA (ETDRS letter) R=.9149845 R^2=.83719655 R^2=.81006264 F(4.24)=30.854 p<.00000 Std. estim. error: 8.0201				
	b*	SD b*	b	SD b	p
			56.86	8.44	0.000001
os/is line	0.67	0.10	24.54	3.78	0.000001
SMD	0.26	0.08	12.71	4.26	0.006518
CRT6000 preop.	–0.28	0.10	–0.05	0.01	0.008142
VMT	–0.17	0.08	–7.55	3.72	0.054130

Table 3. Multiple regression analysis evaluating the relationship of preoperative BCVA, panretinal photocoagulation (PRP), presentation of VMT and preoperative central retinal thickness 1000 µm (CRT 1000) and with a chance in postoperative BCVA on the 12th month.

N=29	BCVA 12 months (ETDRS letter) on the 12th month R=.87364503 R^2=.76325564 R^2=.72021121 F(4.22)=17.732 p<.00000 Std. estim. error: 7.8499				
	b*	SD b*	b	SD b	p
			65.16	10.24	0.000002
Preop. BCVA	0.42	0.14	0.34	0.11	0.006477
PRP	–0.24	0.11	–7.35	3.46	0.045453
VMT	–0.29	0.11	–9.87	3.97	0.021139
Preop. CRT 1000	–0.24	0.12	–0.02	0.01	0.073487

Postoperatively, 28 injections of bevacizumab into the vitreous cavity of 14 eyes were reported (max. 3 injections per eye). Focal macular laser was applied at 1 week after injection. In 2 eyes (6.89%) an additional 25-gauge vitrectomy with drainage of the fluid from the macular cyst was performed.

Preoperative BCVA was positively associated with preservation of the line between outer/inner segments of the photoreceptors (b=0.67; p=0.000001) and the serous macular detachment (b=0.26; p=0.0065) in the foveal region in OCT imagining. Negative factors were presentation of vitreomacular traction (b=–0.17, p=0.05) and greater preoperative central retinal thickness (CRT) in the 6000 µm zone (b=–0.28; p=0.008) (Table 2).

Preoperative BCVA was a statistically significant positive factor for prognosis of BCVA at 12th month follow-up (b=0.42; p=0.0064), the negative factors were previous panretinal photocoagulation (b=–0.24; p=0.045), presentation of vitreomacular traction (b=–0.29; p=0.02) and greater central retinal thickness in region 1000 µm (b=–0.24; p=0.07) (Table 3).

Greater visual acuity improvement occurred in eyes with worse baseline acuity (b=–1.01; p=0.00001). The presentation of vitreomacular traction (b=–0.38; p=0.02), previous panretinal photocoagulation (b=–0.31, p=0.04) and greater preoperative CRT in the 1000 µm zone (b=–0.31; p=0.07) were negative factors for visual improvement (Table 4).

Greater reduction in central retinal thickness occurred with greater preoperative retinal thickness (b=0.77; p<0.000001) (Table 5). Greater structural improvement was not correlated with greater visual acuity improvement.

Discussion

The accumulation of advanced glycation end-products, retina tissue hypoxia, diabetic disorder of hemostasis leading to the breakdown of the blood-retina barrier, induce diabetic retinopathy [17–19] and pathological processes in the vitreomacular interface [20,21]. Particularly important changes occur in the glial cells of the retina and in the vitreous collagen fibers [22,23]. The pathological process of posterior vitreous detachment leads to vitreoschisis, epiretinal membranes, tractions and macular hole formation. This requires surgical removal of the internal retinal membrane (ILM), guaranteeing the total removal of the epiretinal proliferations, which may improve oxygenation of the inner layers of the retina and stimulates intraretinal gliosis [24].

In 1992 Lewis et al. [14] first reported vitrectomy as an alternative treatment for diffuse DME associated with posterior

Table 4. Multiple regression analysis evaluating the relationship of preoperative BCVA, panretinal photocoagulation (PRP), presentation of VMT, preoperative central retinal thickness 1000µm (CRT 1000) with a chance in improvement of BCVA on the 12th month.

N=29			Improvement of BCVA (ETDRS letter) on the 12th month R=.78178321 R^2=.61118498 R^2=.54049134 F(4.22)=8.6455 p<.00024 Std. estim. error: 7.8499		
	b*	SD b*	b	SD b	p
			65.16	10.24	0.000002
Preop. BCVA	–1.01	0.17	–0.65	0.11	0.000011
PRP	–0.31	0.14	–7.35	3.46	0.045453
VMT	–0.38	0.15	–9.87	3.97	0.021139
Preop. CRT 1000	–0.31	0.16	–0.02	0.01	0.073487

Table 5. Multiple regression analysis evaluating the relationship of preoperative central retinal thickness (CRT 1000), lens status (STK), presentation of VMT and SMD with a chance in central retinal thickness 1000 µm (CRT 1000) reduction on the 12th month.

N=29			Reduction of CRT 1000 (µm) on the 12th month R=.88754522 R^2=.78773662 R^2=.74914316 F(4.22)=20.411 p<.00000 Std. estim. error: 111.24		
	b*	SD b*	b	SD b	P
			–365.00	69.53	0.000029
Preop. CRT 1000	0.77	0.11	1.02	0.14	0.000000
Lens status (STK)	0.22	0.10	110.92	51.55	0.042
VMT	0.15	0.10	78.82	53.10	0.15
SMD	0.14	0.11	79.30	66.50	0.24

hyaloid traction. It was used thereafter for management of DME even in the absence of vitreomacular adhesion [25–28]. In 2007 Patel et al. [29], in a prospective, comparative study of PPV with and without ILM peeling for diffuse clinically significant macular edema, reported better structural improvement but with limited visual improvement after ILM peeling compared with leaving ILM. In our opinion, PPV with separation of the posterior hyaloid without ILM peeling in eyes with diabetic macular edema relieves only antero-posterior tractions and edema resolved for a limited time because of an epimacular membrane formation. Vitrectomy including removal of the ILM leads to resolution of diffuse diabetic macular edema and improvement of visual acuity, and prevents subsequent epiretinal membrane formation. Complete release of tractional forces and inhibition of cellular reproliferation seem to be prudent in the eyes of patients with diabetes. We suggest that retinal traction by the posterior hyaloid membrane is involved in the pathogenesis of cystoid changes in diabetic patients. In our study we demonstrated the presence of epimacular membranes in 17 eyes (53%) and vitreomacular tractions in 9 eyes (28%), and in the others 12 eyes (37.5%) without evident vitreomacular interface pathology, PVD was present only in 2 eyes (6.25%). These observations demonstrate a strong correlation between an attached posterior hyaloid membrane and the presence of diffuse macular edema. Therefore, we confirmed that retinal traction by the posterior hyaloid membrane is involved in the pathogenesis of cystoid changes in diabetic patients. Furthermore, the evidence that vitrectomy produces improved retinal oxygenation [30], taken together with the evidence that increased oxygenation can reduce DME [31], suggests an additional physiologic advantage potentially conferred by vitrectomy. Removed ILM contains a part of the Müller cell end-feet and the horizontal gliosis [32]. It is likely that the proliferation of GFAP-stained gliofibrils, observed in damaged Müller cells, preserves the blood-retinal barrier, reinforces architectural cohesion, and opposes the installation of the edema. Therefore, we confirmed the hypothesis that combined triple therapy was effective for decreasing macular thickness and improvement of vision for eyes with diffuse diabetic macular edema. In 2007, Kang et al. [33] reported on triple therapy, which included vitrectomy with subsequent triamcinolone injection into the vitreous cavity, and macular laser photocoagulation. The major adverse events after this therapy included the development of nuclear sclerotic cataracts (8 among 12 phakic eyes) and elevation of intraocular pressure (8 among 24 eyes). In our opinion, this strategy may facilitate early recovery of vision, but the long-term outcomes may be improved by additional lens exchange to eliminate cataract formation. Anti-VEGF usage instead of triamcinolone can prevent the intraocular pressure from increasing.

After vitrectomy, clearance is accelerated and VEGF concentrations are reduced (34). Therefore, we performed injections of bevacizumab only a few days before macular grid/focal photocoagulation in cases of macular edema recurrence during follow-up. We observed resolution of diabetic macular edema and improvement of visual acuity in post-vitrectomized eyes and occasionally we observed an effect on the non-injected fellow eyes. This fact confirms the systemic action of bevacizumab after vitreous cavity delivery and seems to be correlated with the intensity of the hyperfluorescence by fluorescein angiography.

Our study confirms observations that visual acuity is positively associated with preservation of the line between outer/inner segments of the photoreceptors [35]. An interesting observation is, that serous retinal detachment in the macular area positively correlated with preoperative visual acuity. This may be due to the preservation of the integrity of the synaptic connections in the neurosensory retina, raised in contrast to the swollen tissue, where there is a separation of retinal layers by accumulation of the edematous fluid. The negative factors were presentation of vitreomacular traction (VMT) and greater preoperative retinal thickness in the 6000 μm zone, which correlated with enlargement of the foveal avascular zone (FAZ) ($r=0.66$, $p=0.00008$). This fact confirms observations of Pendergast [36] that eyes with macular ischemia tended to respond less favorably to vitrectomy than eyes lacking this characteristic. Preoperative BCVA was a statistically significantly positive factor for prognosis of final BCVA; but on the other hand, greater visual acuity improvement occurred in eyes with worse baseline acuity. Pathological change on OCT, manifested as a loss of the hyperreflective line between outer/inner segments of the photoreceptors in the foveal region, influences worse postoperative visual acuity than in patients without this disorder. Therefore, it is important to perform a careful selection of eyes based on fluorescein angiography and OCT examination and start treatment before development of those irreversible changes of the retinal structure in the foveal region. Careful selection of eyes with favorable preoperative clinical characteristics may improve surgical outcomes. Covariates found to be negative factors for postoperative visual acuity were previous panretinal photocoagulation and greater central retinal thickness in the 6000 μm region. It is possible that panretinal photocoagulation destroying retinal pigment epithelium deprived the retinal tissue of PEDF. The glycoprotein secreted by the RPE cells with neuroprotective and differentiation properties protects against photoreceptor degeneration [37]. RPE destruction dramatically altered the ultrastructure and biosynthetic activity of Müller cells; Müller cells failed to form adherens junctions with photoreceptors. PEDF prevented the degenerative glial response; Müller cells are ultrastructurally normal and form junctional complexes with photoreceptors. Therefore, cytoprotection of RPE is essential for preservation of visual function. Macular laser treatment induces intraretinal gliosis due to excitation of the Müller's cells. This counteracts accumulation of the intraretinal fluid and strengthens the glia-photoreceptor mosaic via creation of lateral connections between the Müller's cells and photoreceptors. In cases where the reaction to the laser treatment is not sufficient, gliosis should be stimulated by removal of ILM, which results in micro-injuries of the external parts of the Müller's cells and triggers their restorative reactions.

Conclusions

The obtained results of functional improvement, reduction of central retinal thickness, and the removal of vitreoretinal interface pathology suggest that the combined therapy is effective and, if performed early, gives very good functional and structural results. The visual functionality of eyes with diabetic maculopathy had statistically significant improvement in all periods of observation, with an upward trend over time. This method has a high therapeutic value as it improves the treatment results of the currently practiced monotherapies and combination therapies [38]. This combined treatment can be an effective method of restoring macular structure and may improve visual acuity for eyes with persistent DME after laser photocoagulation, even in eyes without vitreomacular traction. If we want to treat diabetic maculopathy more effectively, it is necessary to replicate the 2 components of a physiological PVD: gel liquefaction and vitreo-retinal dehiscence. Pharmacologic therapy with use of anti-VEGF agents supports short-term tightness of the blood-retina barrier in the perioperative period and reduces macular thickness before focal/grid laser treatment. This not only facilitates surgery, but, if performed early in the natural history of the disease in conjunction with laser treatment, it should prevent progress of the focal macular edema and may delay surgical treatment. Unfortunately, in the absence of posterior vitreous detachment, pathological changes on the vitreomacular interface need en-block removal of tractions, epimacular proliferations and thickened ILM. Complete removal of ILM reduces the risk of horizontal proliferation on the retina, while it induces intraretinal-vertical gliosis. The existence of these 2 gliosis types was shown in the research of Yvette i Dieter Ducournau [39]. The horizontal gliosis is connected to proliferation of astrocytes and Müller's cells on the internal surface of the retina that subsequently leads to formation of epiretinal membranes. Vertical gliosis is connected to hypertrophy of Müller's cells. Our aim is induction of vertical gliosis, while minimizing the gliosis on the retina's surface. The effect of reactive, vertical gliosis after ILM removal allows for maintaining the blood-retina barrier and prevents macular edema installation. Wolf et al. [40] performed retinal histology via electron microscope after the internal limiting membrane was removed and demonstrated that a significant part of the Müller's cells endings had been damaged. Their main bodies, however, did not show signs of damage. It is likely that the proliferation of GFAP-stained gliofibrils, observed in damaged Müller's cells, preserves the blood-retinal barrier, reinforces architectural cohesion, and opposes the installation of the edema. Without question, a serious flaw connected with leaving parts of the ILM during vitrectomy is increased risk of developing secondary macular pucker syndrome.

Simultaneous endophotocoagulation based on an angiography map allows for the elimination of areas with capillary nonperfusion, which are a source for the synthesis of vascular endothelial growth factor (VEGF). The intraoperative use of anti-VEGF reduces the risk of an increase of the macular edema in the perioperative period. The surgical trauma may increase the macular edema; therefore, during this critical phase it is advisable to utilize a protective anti-VEGF therapy to reduce the edema. As the intraretinal gliosis is a slow process, the anti-VEGF injections must be continued

in the meantime in order to facilitate the approximation of separated retinal layers that will result in restoration of synaptic connections between neurons. This offers a "therapeutic window" for both surgical and laser procedures in case of the recurrence of macular edema after surgery.

The limitation of the present study is that it included relatively small study numbers that may influence the statistical results, and that it lacked a control group. Larger prospective case series are needed to further evaluate the effect of triple therapy on diffused diabetic macular edema (DDME).

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