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Comparative Evaluation of Combined Therapy for Post-Thrombotic Retinopathy: Impact on Hypoxia Index and Retinal Parameters

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Introduction. *Post-thrombotic retinopathy (PTR) is one of the most common retinal vascular diseases, complicated by the development of macular edema (ME) in 60-70% of patients. The main treatment methods include anti-VEGF therapy and laser photocoagulation; however, their efficacy is limited, and anatomical improvement does not always correspond to the restoration of retinal functional status. Therefore, there is a need to develop methods that allow for the objective assessment of hypoxic changes during therapy.*

Purpose*. To compare the effectiveness of combined anti-VEGF therapy and subthreshold micropulse laser treatment using a yellow laser (577 nm) versus anti-VEGF monotherapy in patients with post-thrombotic retinopathy, focusing on changes in the hypoxia index (Cg) as a functional biomarker, along with anatomical and visual outcomes.*

Methods*. The study included 60 patients (66 eyes) with PTR, divided into two groups: combined therapy (anti-VEGF + micropulse laser, 31 eyes) and anti-VEGF monotherapy (35 eyes). All patients underwent ophthalmological examination, optical coherence tomography (OCT), and electroretinography (ERG) with calculation of the Cg. Assessments were conducted at baseline and at 3, 6, and 12 months.*

Results. *By the twelfth month, central retinal thickness in the combined therapy group had decreased to 310±25 μm compared to 360±29 μm in the monotherapy group (p=0.006). Best-corrected visual acuity improved to 0.5±0.03 versus 0.33±0.03, respectively (p=0.003). The hypoxia index (Cg) significantly decreased from 5.6±0.32 to 5.27±0.25 in the combined therapy group, while no significant changes were observed in the monotherapy group (p=0.003).*

Conclusion*. Combined therapy using an anti-VEGF agent and subthreshold micropulse laser treatment promotes more pronounced anatomical and functional recovery of the retina in PTR. The use of the hypoxia index (Cg) provides an objective evaluation of therapy efficacy at the metabolic level and may be recommended for clinical practice.*

Key words:
retinal vein occlusion; post-thrombotic retinopathy; macular edema; anti-VEGF therapy; micropulse laser; hypoxia index

Introduction. Post-thrombotic retinopathy (PTR), which develops in cases of retinal vein occlusion (RVO), is one of the most common vascular retinal disorders, second only to diabetic retinopathy (DR) in prevalence. According to epidemiological studies, RVO is diagnosed annually in 0.5–1.2% of the adult population over 40 years of age, contributing significantly to the overall causes of secondary vision loss [1, 2, 3]. One of the main complications of PTR is macular edema (ME), which develops in 60-70% of patients and serves as a leading factor in persistent visual acuity reduction [4, 5].

Currently, the primary treatment methods for ME in PTR include anti-VEGF injections and laser photocoagulation. Anti-VEGF therapy enables disease stabilization and temporary vision improvement; however, a significant proportion of patients experience recurrence or persistent macular edema requiring repeated interventions. Although standard laser photocoagulation can reduce edema, it is associated with damage to the neurosensory structures of the retina, limiting its use [2, 4, 5]. Thus, there is a need to develop effective treatments that are less invasive and capable of providing long-term stabilization of the retinal condition.

One promising approach is the use of subthreshold micropulse laser therapy, in which laser energy is delivered fractionally, minimizing the risk of thermal tissue damage [6]. Numerous studies [7–11] have demonstrated that micropulse laser therapy stimulates reparative processes in the retinal pigment epithelium, promotes the restoration of the barrier function, and reduces macular edema without the formation of visible burns. However, despite these positive outcomes, the effect of micropulse laser therapy on retinal metabolic processes remains insufficiently studied.

The traditional assessment of treatment efficacy is based on optical coherence tomography (OCT) data, reflecting morphological changes in the retina [5]. However, anatomical improvement does not always correlate with the restoration of neuronal functional activity. In this regard, there is a need for additional objective methods to assess the functional status of the retina. One such method is the calculation of the hypoxia index (Cg) based on electroretinography (ERG) data, which allows for the early detec-

tion of retinal dysfunction and monitoring of the metabolic recovery dynamics in response to therapy [12–14].

The aim of this study is a comparative evaluation of the efficacy of combined anti-VEGF therapy and subthreshold micropulse laser treatment using a yellow laser (577 nm) in patients with post-thrombotic retinopathy, with an emphasis on the dynamics of the hypoxia index (Cg) as a key marker of the functional state of the retina, as well as an analysis of associated anatomical and visual characteristics compared to traditional anti-VEGF monotherapy.

Materials and Methods

Study design. This was a prospective, comparative, observational study conducted at the Laser Department of the Republican Specialized Scientific and Practical Medical Center of Eye Microsurgery in Tashkent, Uzbekistan, between February 2023 and February 2024.

Clinical Material Characteristics

Inclusion criteria: patients aged 40–70 years with a diagnosis of ME secondary to central retinal vein occlusion (CRVO) or branch retinal vein occlusion (BRVO) confirmed by OCT.

Exclusion criteria: patients with previously treated ME (panretinal photocoagulation or anti-VEGF), prior intraocular surgery in the last 6 months, uncontrolled systemic disease, and advanced proliferative retinopathy.

The study included 60 patients (66 eyes) diagnosed with post-thrombotic retinopathy (PTR). The cohort consisted of 34 men and 26 women. The mean age of the patients was 51,3±4,7 years. Of the 66 eyes included, 38 (57.6%) had CRVO and 28 (42.4%) had BRVO. The duration since the episode of CRVO or BRVO ranged from 1 to 4 months.

Group Formation. The allocation was non-randomized but matched for age, sex, and disease severity. Group 1 (28 patients, 31 eyes) received combined treatment - intravitreal injection of an anti-VEGF agent followed by subthreshold micropulse laser therapy using a yellow laser (577 nm). Group 2 (32 patients, 35 eyes) received only anti-VEGF therapy without laser treatment.

Description of Combined Treatment. Subthreshold micropulse laser therapy was performed using the Supra 577 nm laser system (Quantel Medical, France) in micropulse mode. The Volk Area Centralis lens (Volk Optical, USA) was used during procedure. Treatment parameters included a wavelength of 577 nm, power of 250-400 mW, exposure time of 0,03 ms, duty cycle of 10–12%, and a spot diameter of 300-350 μm. Prior to therapy, individual pulse power titration was performed outside the vascular arcade, starting at 50 mW and increasing until a grade 1 burn according to F. L'Esperance's classification (1983) was achieved. After reaching the threshold grade 1 burn to determine the titration point, the power was reduced by approximately 50% for the subthreshold micropulse treatment, which was performed in a true subthreshold mode (without visible burns), in line with established protocols. The micropulse laser therapy session was conducted three days after the first anti-VEGF injection. Laser treatment was repeated in 7 eyes at the 3-month visit based on persistent edema.

Anti-VEGF Therapy Protocol. In all cases, the anti-VEGF agent "Визкью" ("Beovu") (Novartis, Switzerland; international nonproprietary name: brolucizumab) was used. All patients received a loading dose of three monthly brolucizumab injections (one every 4 weeks). After the loading phase, additional injections were administered as needed based on OCT findings, following a pro re nata (PRN) regimen. The drug was administered intravitreally at a dose of 0.2 mL (2.0 mg) under sterile operating room conditions. In the combined therapy group, the anti-VEGF agent was used as loading therapy prior to laser treatment.

Examination Methods. All participants underwent standardized assessments at baseline, 3, 6, and 12 months, including best-corrected visual acuity (BCVA), optical coherence tomography (OCT), and electroretinography (ERG) for calculation of the glial hypoxia index (Cg). Optical coherence tomography (OCT) of the macular area was performed using a Swept Source OCT system, DRI OCT Triton (Topcon, Japan). OCT assessments were standardized by performing all scans on the same device.

OCT Parameter Analysis. The analysis included measurement of central retinal thickness, detection of cystoid changes, identification of subretinal fluid, evaluation of hyperreflective foci, assessment of disorganization of retinal inner layers (DRIL), and measurement of choroidal thickness in subfoveal, nasal, and temporal zones.

Methods of Functional Retinal Assessment. Electroretinography was conducted using the Neuro-ERG system (Neurosoft, Russia) in accordance with ISCEV standards. To objectively assess functional changes in the retina, electroretinography (ERG) was performed, including recording of the b-wave of full-field ERG and rhythmic ERG at 12 Hz (RERG 12 Hz). Based on these recordings, the glial hypoxia index (Cg) was calculated using the formula:

Cg = Amplitude of full-field ERG b-wave / Amplitude of RERG 12 Hz,

where Cg represents the glial hypoxia index. The b-wave amplitude of full-field ERG reflects the activity of Müller glial cells and bipolar neurons, while RERG 12 Hz reflects the activity of bipolar cells. An increase in Cg indicates a decrease in neuronal activity relative to an increase in glial cell activity, suggesting the development of retinal hypoxia. During hypoxia, neurons – particularly bipolar cells – are the first to be affected. Glial cells then begin to compensate for the damage. Thus, early alterations in the b-wave and RERG 12 Hz amplitude ratio serve as a marker of hypoxia.

Statistical Analysis. Statistical analysis was performed using SPSS software version 26.0. Normality of data distribution was assessed using the Shapiro-Wilk test. Quantitative variables between groups were compared using the Student's t-test or the Mann-Whitney U test, depending on data distribution. Categorical variables were compared using the χ^2 test. Differences were considered statistically significant at $p<0,05$.

This study was approved by the local bioethics committee (Protocol №3, 02.02.2023). Written informed consent was obtained from all participants prior to inclusion in the study, in accordance with the Declaration of Helsinki.

Results

At baseline, the mean central retinal thickness was comparable between the groups (p=0.278). By the third month of treatment, a significant reduction in retinal thickness was observed in both groups; however, the effect was more pronounced in the combined therapy group (p=0.015). This trend persisted at six months (p=0.009) and twelve months (p=0.006). Cystoid changes persisted at twelve months in 25,8% of patients in Group 1 and in 42.9% of patients in Group 2 (p=0.042). The frequency of subretinal fluid presence was also lower in the combined therapy group (p=0.038). The proportion of patients with signs of disorganization of the retinal inner layers (DRIL) at twelve months was lower in Group 1 (19.3% versus 31.4%), although the difference did not reach statistical significance (p=0.092). Choroidal thickness did not change significantly over time in either group (Table 1).

The amplitude of the full-field ERG b-wave in the combined therapy group increased from 214±12 to 247±12 μV after 12 months, which was statistically significant compared to the monotherapy group (p=0.001). Similarly, the amplitude of the rhythmic ERG at 12 Hz (RERG 12 Hz) was significantly higher in Group 1 throughout the observation period (p=0.001). The hypoxia index (Cg) decreased more markedly in the combined therapy group (from 5.6±0.32 to 5.27±0.25), whereas in Group 2, virtually no changes were observed. The difference between the groups was statistically significant at all observation points starting from the third month (p<0.05) (Table 2).

At baseline, the mean best-corrected visual acuity (BCVA) was comparable between the groups (p=0.601). After three months, a significant improvement in BCVA was observed in both groups; however, the results in the combined therapy group were significantly higher (p=0.020). This trend persisted and became more pronounced during subsequent follow-up visits. By the sixth month, the BCVA in Group 1 reached 0.42±0.03, which was significantly higher than in Group 2 (p=0.007). By the twelfth month, further improvement was noted in the combined therapy group, reaching 0.5±0.03 compared to 0.33±0.03 in Group 2 (p=0.003). Within-group analysis showed that in Group 1, the improvement in BCVA was statistically significant at all time points (p<0.01). In Group 2, although a positive trend was observed, statistical significance was only achieved at earlier stages of treatment, with no significant differences noted at six and twelve months (p>0.05) (Table 3).

Discussion

Post-thrombotic retinopathy (PTR) represents a serious complication of retinal vascular diseases, leading to the development of macular edema (ME) and impaired visual function. Despite the widespread use of anti-VEGF therapy, the issue of persistent or recurrent ME remains relevant, particularly against the background of chronic retinal hypoxia and disorganization of its inner structure. Early diagnosis of tissue metabolic disturbances, particularly hypoxia, is a crucial area for improving treatment efficacy and predicting outcomes.

In this study, a combined therapy approach was proposed to address this issue, integrating anti-VEGF agents with subthreshold micropulse laser treatment using a yel-

Table 1. Dynamics of OCT Parameters

Parameter	Time Point	Group 1 (31 eyes)	Group 2 (35 eyes)	p Between Groups	p Within Group 1	p Within Group 2
Central retinal thickness (μm), M±SD	Baseline	495±35	485±38	0.278	—	—
	3 months	360±30	395±32	0.015	0.014	0.087
	6 months	330±28	375±30	0.009	0.007	0.079
	12 months	310±25	360±29	0.006	0.004	0.072
Presence of cystoid changes, n (%)	Baseline	27 (87.1%)	29 (82.8%)	0.531	—	—
	12 months	8 (25.8%)	15 (42.9%)	0.042	0.011	0.056
Presence of subretinal fluid, n (%)	Baseline	14 (45.1%)	17 (48.5%)	0.770	—	—
	12 months	3 (9.7%)	8 (22.8%)	0.038	0.009	0.049
DRIL, n (%)	Baseline	13 (41.9%)	14 (40%)	0.827	—	—
	12 months	6 (19.3%)	11 (31.4%)	0.092	0.058	0.088
Choroidal thickness (μm), M±SD	Baseline	260±18	265±20	0.456	—	—
	12 months	250±17	255±19	0.462	0.219	0.345

Notes: M±SD — mean value ± standard deviation; n (%) — number of cases and percentage. p-values < 0,05 are considered statistically significant and are shown in bold. For quantitative variables, the Student's t-test or Mann–Whitney U test was used depending on data distribution. For categorical variables, the χ^2 test or Fisher's exact test was applied.

Table 2. Dynamics of ERG Functional Parameters.

Parameter	Time Point	Group 1 (31 eyes)	Group 2 (35 eyes)	p Between Groups	p Within Group 1	p Within Group 2
Amplitude of full-field ERG b-wave (μV), M±SD	Baseline	214±12	212±13	0.482	—	—
	3 months	232±11	220±14	0.019	0.014	0.085
	6 months	241±10	223±13	0.004	0.006	0.074
	12 months	247±12	222±15	0.001	0.004	0.068
Amplitude of RERG 12 Hz (μV), M±SD	Baseline	38.2±2.8	37.5±3.0	0.378	—	—
	3 months	43.2±3.5	39.1±3.2	0.007	0.012	0.081
	6 months	45.6±3.2	39.5±3.0	0.002	0.006	0.077
	12 months	46.8±3.1	39.0±3.1	0.001	0.004	0.075
Hypoxia index (Cg), M±SD	Baseline	5.60±0.32	5.65±0.34	0.514	—	—
	3 months	5.37±0.28	5.63±0.30	0.018	0.015	0.093
	6 months	5.28±0.26	5.64±0.29	0.005	0.008	0.088
	12 months	5.27±0.25	5.69±0.30	0.003	0.006	0.084

Notes: M±SD — mean value ± standard deviation; n (%) — number of cases and percentage. p-values < 0.05 are considered statistically significant and are shown in bold. For quantitative variables, the Student's t-test or Mann–Whitney U test was used depending on data distribution. For categorical variables, the χ^2 test or Fisher's exact test was applied.

Table 3. Dynamics of Best-Corrected Visual Acuity (BCVA)

Time Point	Group 1 (31 eyes)	Group 2 (35 eyes)	p Between Groups	p Within Group 1	p Within Group 2
Baseline	0.17±0.02	0.16±0.02	0.601	—	—
3 months	0.35±0.03	0.26±0.03	0.020	0.008	0.072
6 months	0.42±0.03	0.30±0.03	0.007	0.005	0.059
12 months	0.50±0.03	0.33±0.03	0.003	0.003	0.051

Notes: M±SD — mean value ± standard deviation; n (%) — number of cases and percentage. p-values < 0.05 are considered statistically significant and are shown in bold. For quantitative variables, the Student's t-test or Mann–Whitney U test was used depending on data distribution. For categorical variables, the χ^2 test or Fisher's exact test was applied.

low laser (577 nm). Such a combination allows simultaneous suppression of pathological neovascular activity and stimulation of reparative processes in the retina by activating cells without causing tissue damage. A key element of the study was the use of the hypoxia index (Cg), reflecting the ratio of neuronal to glial activity in the retina, as a sensitive marker of early circulatory disturbances and treatment efficacy.

In the development of ME due to PTR, Müller glial cells undergo mechanical stretching, disrupting their fluid-pumping and barrier-maintaining functions. This initiates processes of activation and reactive gliosis, leading to pathological remodeling of glial cells. Prolonged damage results in degeneration of Müller cells and impairment of aquaporins (AQP4) - special membrane proteins responsible for water transport. As a consequence, the retina loses its ability to normalize edema, leading to persistent edema and disruption of the inner blood-retinal barrier, ultimately resulting in chronic ME [12-14]. These findings confirm that the Cg index can be used as an early indicator of retinal deterioration in PTR and ME and as a marker for therapy efficacy.

The results demonstrated that combined therapy led to a more pronounced reduction in central retinal thickness, a decrease in the frequency of cystoid changes and subretinal fluid, as well as a significant improvement in best-corrected visual acuity. Critically, the hypoxia index (Cg) decreased significantly faster and more consistently in the combined therapy group compared to the monotherapy group, indicating more effective normalization of metabolic processes in the retina. The absence of significant changes in choroidal thickness suggests that the therapy primarily affected the inner retinal layers.

Our findings are consistent with previous studies showing the efficacy of micropulse laser therapy in various forms of macular edema. For instance, studies by Nichani et al. (2024) [15] and Hosoya et al. (2024) [6] reported that subthreshold laser therapy reduces edema severity without inducing visible retinal damage. However, our study was the first to systematically assess the impact of combined

therapy on the hypoxia index, providing a deeper understanding of the pathogenetic mechanisms underlying retinal improvement.

Thus, the use of the hypoxia index (Cg) as a functional marker allows for an objective evaluation of the efficacy of combined treatment for post-thrombotic retinopathy. The data obtained emphasize the importance of a comprehensive approach aimed not only at eliminating anatomical manifestations of the disease but also at restoring the functional state of the retina at early stages of hypoxic injury.

Conclusion. Combined therapy, including an anti-VEGF agent and subthreshold micropulse laser treatment, resulted in significant improvements in both anatomical and functional parameters in patients with post-thrombotic retinopathy. By twelve months, central retinal thickness had decreased to 310±25 μm in the combined therapy group compared to 360±29 μm in the monotherapy group (p=0.006), and best-corrected visual acuity improved to 0.50±0.03 versus 0,33±0,03, respectively (p=0.003). The hypoxia index (Cg) in the combined therapy group significantly decreased from 5.6±0.32 to 5.27±0.25 (p=0.003), reflecting an improvement in the functional state of the retina. These findings confirm the high efficacy of the combined approach and the feasibility of using the hypoxia index as an early, objective marker for evaluating treatment outcomes.

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Data Availability Statement. *The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.*

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