

Intraocular cytokines in retinal vein occlusion and its relation to the efficiency of anti-vascular endothelial growth factor therapy

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Purpose: To analyze the change in the concentration of intraocular cytokines (ICs) in patients with retinal vein occlusion (RVO) before and after intravitreal ranibizumab therapy (IVR), and to find the correlations of IC with clinical activity of RVO and efficiency of treatment. **Materials and Methods:** Forty-four patients aged 46–79 years old (mean age: 60.7 ± 7.5 years old) with RVO and macular edema (18 patients – with central RVO, 26 – with branch RVO) treated with IVR were included into the study. The concentrations of 27 cytokines were simultaneously measured in aqueous humor by flow fluorometry using Bio-Plex Pro Human Cytokine Panel, 27-Plex (Bio-Rad Laboratories, USA) at baseline and after the first IVR. Control group consisted of 20 age-matched patients. **Results:** The levels of 11 cytokines (vascular endothelial growth factor [VEGF], receptor antagonist interleukin-1, interleukin-6 [IL-6], IL-8, IL-9, IL-10, IL-12r70, IL-13, IL-15, monocyte chemoattractant protein-1 [MCP-1], regulated on activation, normal T expressed and secreted) were significantly ($P < 0.05$) different compared to control and significantly ($P < 0.05$) changed after IVR both in central and branch RVO. The patients were divided into two groups: the first – “effective” and the second – “partially effective” therapy. The second group characterized by the higher concentrations of VEGF, IL-8, IL-10, IL-17, and MCP-1 at baseline compared to the first group. **Conclusion:** The patients with RVO were characterized by the increased levels of VEGF and other pro- and anti-inflammatory cytokines and chemokines. Aqueous concentration of cytokines were different in patients with central and branch RVO and significantly changed after IVR. Insufficient response to IVR was associated with activation of immune-inflammatory processes.

Key words: Anti-vascular endothelial growth factor treatment efficiency, aqueous humor cytokines, ranibizumab, retinal ischemia, retinal vein occlusion

Retinal vein occlusion (RVO) is a serious and widespread peripheral retinal vascular disease characterized by intravascular or extravascular obstruction leading to the formation of retinal hemorrhages, exudation of fluid, and ischemia of different severity. Such vascular damage is accompanied by a cascade of cellular and inflammatory response which hinders normal interaction of regulatory mechanisms in damaged tissue. An imbalance of inflammatory and angiogenic cytokines occurs and possibly defines anatomic and functional abnormalities of the eye.^[1,2]

Cytokines are a group of mediators involved in the regulation of defense reactions to pathogen invasion or solution of continuity of the tissue. First of all, cytokines regulate development of defense reactions in tissues involving different types of blood cells, endothelial cells, connective tissue cells, and glial cells.^[3] Local immunity is developed by forming a

typical inflammatory response with its usual signs – swelling and functional impairment.

Despite intensive studies of the role of cytokines in different inflammatory and vascular reactions of the eye, now there is no precise information on the quantity of cytokines at different phases of postocclusive retinal damage. Moreover, there is no data to our knowledge on the change in intraocular cytokines (ICs) concentrations during the course of anti-angiogenic treatment with ranibizumab.

The purpose of our study is to analyze the change in the concentration of ICs in patients with RVO before and after intravitreal ranibizumab (IVR) therapy, and to find the correlations of IC with clinical activity of RVO and efficiency of treatment.

Materials and Methods

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the

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institution. All patients signed an informed consent form prior to participation in the study.

Subjects

Forty-four patients (10 men, 34 women) with RVO aged 46–79 (mean age: 60.7 ± 7.5 years old), 18 of them (5 men, 13 women) had the diagnosis of central RVO (CRVO) and 26 of them (6 men, 20 women) – branch RVO (BRVO). The control group included 20 patients (11 men, 9 women) with an early stage of uncomplicated cataract with relatively high best-corrected visual acuity, scheduled for cataract surgery, aged 49–71 (mean age 60 ± 6.1 years old).

The inclusion criterion was macular edema in the setting of CRVO or BRVO with the duration from 3 weeks to 3 months. Neovascularization of the retina or iris, secondary glaucoma, or previous surgical or laser were considered the exclusion criteria.

To define prognostic criteria of effectiveness for the anti-vascular endothelial growth factor (VEGF) treatment, after ranibizumab treatment, all patients were divided into two groups – with “effective” and “partially effective” therapy (or “sufficient” and “insufficient” response). The criteria of effective therapy were increase in visual acuity by more than 0.05 (20/400), decrease in central retinal thickness by more than twice, and improvements in the results of electrophysiological examination (increases in a- and b-wave amplitudes and oscillatory potentials [OPs]) 1 month after IVR. The criteria of insufficient response were decrease in visual acuity, insignificant changes in central retinal thickness and in electroretinography (ERG) data. The first group (“effective”) included 30 patients (9 with CRVO and 21 – with BRVO). The second group (“partially effective”) included 14 patients (9 – with CRVO and 5 – with BRVO).

Samples collection and laboratory assessment

All patients with RVO were treated with ranibizumab (0.5 mg). The collection of aqueous humor (100–150 mL) was performed immediately before the ranibizumab injection by 30-gauge-needle syringe. After that, the fluid was placed in a sterile test tube and placed in a freezer under -80°C before the laboratory analysis. 1 month later, the intraocular fluid of all patients was sampled again before the IVR. Intravitreal injections as well as the collection of aqueous humor samples were performed by the same surgeon.

Aqueous humor sampling in the control group was carried out by the same technique right before the cataract surgery.

Multiplex proteomic analysis was used to measure the level of cytokines in the aqueous humor samples. The concentration of 27 human cytokines (interleukin-1 β [IL-1 β], receptor antagonist IL-1 [RAIL-1], IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17A, eotaxin, fibroblast growth factor [FGF]-basic, granulocyte-colony stimulating factor [G-CSF], granulocyte macrophage-CSF [GM-CSF], interferon- γ [IFN- γ], induced protein-10, monocyte chemotactic protein-1 [MCP-1], macrophage inflammatory protein-1 α [MIP-1 α], MIP-1 β , platelet derived growth factor-BB [PDGF-BB], regulated on activation, normal T expressed and secreted [RANTES], tumor necrosis factor- α [TNF- α], and VEGF) was measured by flow-through fluorometry^[4] using Bio-Plex Protein Assay

System, Bio-Rad (USA) according to the manufacturer’s instruction.

Ophthalmological examination

Ophthalmological examination included standard methods of diagnosis (visual acuity measurement and ophthalmoscopy) as well as examinations of morphology and retinal functions optical coherence tomography (Cirrus HD-OCT, Carl Zeiss Meditec Inc., USA), fluorescein angiography (TRC-50DX, Topcon, Japan), ultrasound (Voluson 730 Pro, General Electric, USA), and computer perimetry (Twinfield, Oculus, USA) and ERG (EP-1000 PC, Tomey, Japan).

Statistical analysis

The statistical data on all the studied parameters were represented as the mean value \pm standard deviation. The difference between the treatment groups and control group was defined using Mann–Whitney U-test, and the difference between groups before and after treatment was defined by Wilcoxon W-test. In addition, for definition of the most informative features of difference in the groups, discriminant analysis with the definition of F-criterion was carried out:

$$F = \frac{S_b^2}{S_w^2}$$

where S_b^2 - inter-group variance of the feature; S_w^2 - intra-group variance of the feature. It is evident that the more and the less S_b^2 , the more is the diagnostic relevance of the feature.^[5] Usually, the model includes features where the level of significance according to F-criterion is $P < 0.05$. The critical level of significance (P) upon the examination of statistical hypotheses was 0.05.

All the calculations were made using the program STATISTICA 8.0.550 Portable, StatSoft Inc., USA.

Results

The analysis of 27 cytokines in aqueous humor of the patients in the study and control groups showed statistically significant differences in the concentrations of 11 of them. At baseline, the concentrations of VEGF, pro- and anti-inflammatory cytokines (IL-6, IL-12 and IL-10, IL-13 accordingly), and chemokines (IL-8, MCP-1) were elevated in RVO patients compared to the control group, and the concentrations of RAIL-1, IL-9, and RANTES (T-cell-directed CC chemokine) were significantly lower than the control group [Table 1]. The concentration of IL-15 was increased by 70% in patients with CRVO and by 39% in patients with BRVO ($P < 0.05$).

The study of cytokines in the aqueous humor of RVO patients 1 month after IVR found a significant decrease in concentrations of the majority of cytokines [Table 2]. As expected, after the injection of anti-VEGF agent, the concentration of VEGF has significantly changed with 27-times decrease in CRVO group and 5-times decrease in BRVO group. Moreover, a decrease of concentrations of pro- and anti-inflammatory cytokines (IL-6, IL-12 and IL-10, IL-13, IL-15 accordingly) and chemokines (IL-8, MCP-1) was registered. The concentrations of RAIL-1 and IL-9 also decreased after IVR, despite there were lower concentrations at baseline compared to the control group.

It should be noted that the “sufficient” clinical effect of IVR in the subgroup of BRVO patients was reached

in 81% of cases (21 of 26 patients), whereas in patients with CRVO, the effect of treatment was significantly less (50% or 9 of 18 patients).

Comparative analysis of baseline ophthalmological parameters in RVO subgroups showed that the status of patients with “insufficient” clinical effect was characterized by more significant thickening of foveal and peripapillary retina, depression of a- and b- wave amplitudes, and OPs according to ERG [Table 3].

Comparative analysis of baseline cytokine level in RVO patients showed that patients with “insufficient” clinical effect were characterized by a significant increase not only VEGF, but also chemokines (IL-8, MCP-1) as well as anti-inflammatory cytokines (IL-10, IL-13) [Table 4].

Discriminant analysis

To define the mechanisms of anti-VEGF therapy, it is necessary to find the most informative criteria of difference

Table 1: Concentrations of cytokines in aqueous humor of patients in the study and control groups at baseline

Cytokine (pg/mL)	CRVO (n=18)	BRVO (n=26)	Control (n=20)	P _u
	1	2	3	
VEGF	1725.24±946.95	919.07±533.29	126.61±55.35	1-2<0.01 1-3<0.001 2-3<0.001
RAIL-1	24.41±16.19	26.63±24.99	49.39±34.72	1-3<0.005 2-3<0.005
IL-6	285.73±232.51	65.82±97.46	52.19±34.55	1-2<0.01 1-3<0.01 2-3<0.05
IL-8	181.27±94.79	55.41±60.65	30.1±30.06	1-2<0.01 1-3<0.05 2-3<0.05
IL-9	11.5±10.25	12.39±13.01	24.52±26.19	1-3<0.05 2-3<0.05
IL-10	26.22±23.87	13.04±8.06	5.72±2.27	1-2<0.05 1-3<0.005 2-3<0.005
IL-12p70	171.74±116.71	80.2±48.18	34.32±13.75	1-2<0.01 1-3<0.005 2-3<0.005
IL-13	160.35±153.51	62.54±110.94	13.66±5.61	1-2<0.01 1-3<0.01 2-3<0.01
IL-15	6.4±2.38	5.23±4.28	3.76±1.48	1-3<0.05 2-3<0.05
MCP-1	1309.1±847.1	562.27±441.51	328.19±82.76	1-2<0.005 1-3<0.005 2-3<0.005
RANTES	5.12±5.26	15.37±28.33	25.83±16.89	1-2<0.05 1-3<0.05 2-3<0.05

The data is presented as mean±SD. P - Significance of differences in groups (U - Mann–Whitney U-test). CRVO: Central retinal vein occlusion, BRVO: Branch retinal vein occlusion, SD: Standard deviation, VEGF: Vascular endothelial growth factor, RAIL-1: Receptor antagonist of interleukin-1, IL: Interleukin, MCP-1: Monocyte chemoattractant protein-1, RANTES: Regulated on activation, normal T expressed and secreted

in the examined groups. Multifactorial discriminant analysis of all the features including concentration of examined cytokines and basic ophthalmological parameters in patients with “sufficient” and “insufficient” clinical effect was performed. For calculation purposes, all the patients were divided into four groups: Group 1 - initial status of the patient with subsequent “sufficient” effect of ranibizumab treatment; Group 2 - initial status of patients with subsequent “insufficient” effect of anti-VEGF ranibizumab treatment; Group 3 - status of patients with “sufficient” effect after ranibizumab administration; and Group 4 - status of patients with “insufficient” effect after ranibizumab administration. The scheme for discriminant analysis is as follows [Fig. 1]: In patients within the pairs of groups (1st–2nd and 3rd–4th groups, 1st–3rd and 2nd–4th groups).

The most informative features that allowed us to define the mechanisms of ranibizumab effect in patients with “sufficient” clinical effect with high level of evidence were not only a predictable decrease in VEGF (F = 51.9; P < 0.0001), but also decrease in central retinal thickness (F = 45.3; P < 0.0001) as well as an increase in a- and b-wave amplitudes (F = 8.7; P < 0.006) and OPs (F = 6.9; P < 0.02). Ranibizumab injection also significantly depressed inflammatory cytokines: IL-15 (F = 16.1; P < 0.0005), IL-6 (F = 13.7; P < 0.001), and IL-8 (F = 8.6; P < 0.01).

In cases of “insufficient” clinical response, ranibizumab effect included, besides a decrease in VEGF (F = 87.9; P < 0.0001), only the change in RAIL and IL-12p70 concentrations, which did not result in significant decrease of macular edema and restoration of retinal functions.

A multifactorial discriminant analysis of all the four patient groups was carried out to define mutual similarity and difference of patients’ visual system at baseline and after IVR. The canonical variables (CVs) for each patient that were used for plotting a dot graph [Fig. 2] were found by canonical equation for four groups (n = 88). CV1 – informativeness level 74%, CV2 – informativeness level 24%, and CV3 – informativeness level 2%.

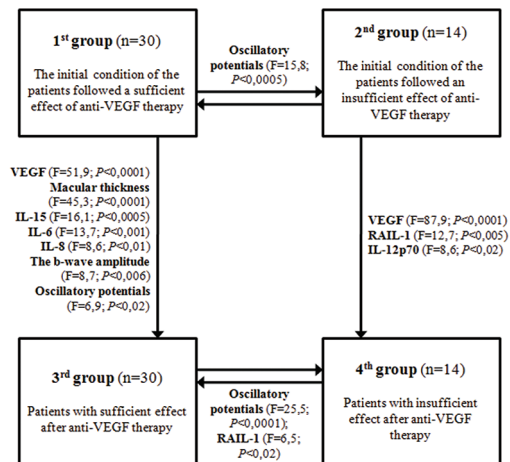


Figure 1: Scheme of differentiation of patients with sufficient and insufficient clinical effect of anti-vascular endothelial growth factor therapy. Patients with “sufficient” and “insufficient” clinical response to intravitreal ranibizumab

Table 2: Concentrations of cytokines in aqueous humor at baseline and 1 month after intravitreal ranibizumab injection

Cytokine (pg/mL)	CRVO before treatment (n=18)	CRVO 1 month after ranibizumab injection (n=18)	BRVO before treatment (n=26)	BRVO 1 month after ranibizumab injection (n=26)	P_w
	1	2	3	4	
VEGF	1725.24±946.95 (378-3713.9)	63.11±55.01 (7.3-166.7)	919.07±533.29 (95-2057)	197.55±271.55 (6.8-866.7)	1-2<0.001 3-4<0.005
RAIL-1	24.41±16.19 (8.7-59.77)	9.69±3.14 (8.7-18.6)	26.63±24.99 (8.7-85.72)	26.47±31.64 (8.4-110.1)	1-2<0.01
IL-6	285.73±232.51 (87.7-822)	61.31±78.13 (3.2-210.2)	65.82±97.46 (0.7-336.1)	25.05±31.13 (0.3-136.9)	1-2<0.005 3-4<0.05
IL-8	181.27±94.79 (47.8-288.4)	77.13±111.63 (4.2-382.2)	55.41±60.65 (13.5-278.6)	47.99±50.74 (4.2-164.7)	1-2<0.01 3-4<0.05
IL-9	11.5±10.25 (1.5-24.5)	7.91±15.89 (1.5-51.3)	12.39±13.01 (1.5-56.5)	9.31±11.08 (1.5-49.4)	1-2<0.05 3-4<0.05
IL-10	26.22±23.87 (9.3-87.7)	5.67±7.45 (0.4-21)	13.04±8.06 (2.2-36.9)	9.38±8.21 (0.6-37.8)	1-2<0.01 3-4<0.05
IL-12p70	171.74±116.71 (72.8-470.2)	23.14±23.42 (7-79.1)	80.2±48.18 (9.9-172.4)	39.06±39.04 (6.4-112.9)	1-2<0.001 3-4<0.01
IL-13	160.35±153.51 (7.9-416.2)	61.31±78.13 (3.2-210.2)	62.54±110.94 (6.8-478.6)	65.72±162.97 (4.2-568.8)	1-2<0.005
IL-15	6.4±2.38 (3.9-12.6)	4.07±4.89 (0.3-17.5)	5.23±4.28 (0.8-20)	2.72±2.51 (0.3-8.1)	1-2<0.05 3-4<0.05
MCP-1	1309.1±847.1 (360.3-3189.6)	659.67±378.64 (112.8-1171.9)	562.27±441.51 (203.8-2227.7)	388.94±253.83 (105-1102.3)	1-2<0.005 3-4<0.005
RANTES	5.12±5.26 (2.7-17.5)	6.91±13.31 (2.7-44.8)	15.37±28.33 (2.7-122.5)	12.01±21.15 (1.2-92)	1-2<0.05 2-3<0.05

The data is presented as mean±SD; minimum-maximum. P - significance of differences in groups (W - Wilcoxon signed-rank test for linked samples). CRVO: Central retinal vein occlusion, BRVO: Branch retinal vein occlusion, SD: Standard deviation, VEGF: Vascular endothelial growth factor, RAIL-1: Receptor antagonist of interleukin-1, IL: Interleukin, MCP-1: Monocyte chemoattractant protein-1, RANTES: Regulated on activation, normal T expressed and secreted

Table 3: Baseline visual acuity, retinal thickness and parameters of ERG in patients with "sufficient" and "insufficient" clinical response to IVR

Parameter	Group 1 (+) (n=30)	Group 2 (-) (n=14)	Control (n=20)	P_u
	1	2	3	
Visual acuity (units)	0.18±0.17	0.05±0.07	0.91±0.11	1-3<0.001 2-3<0.001
Thickness of foveal retina (mcm)	619.21±222.18	872.14±352.07	202.85±22.21	1-2<0.02 1-3<0.05 2-3<0.05
Thickness of peripapillary retina (mcm)	418.69±185.56	719.42±406.02	314.2±33.19	1-2<0.05 1-3<0.01 2-3<0.01
ERG a-wave, amplitude (mkV)	58.59±7.25	46.57±7.47	65.43±12.35	1-2<0.001 1-3<0.005 2-3<0.001
ERG b-wave, amplitude (mkV)	112.82±11.80	96.60±12.20	148.62±16.8	1-2<0.01 1-3<0.005 2-3<0.001
Oscillatory potentials (mkV)	22.21±6.42	10.52±1.17	67.65±12.24	1-2<0.005 1-3<0.001 2-3<0.001

The data is presented as mean±SD. P - significance of differences in groups (U - Mann-Whitney U-test). IVR: Intravitreal ranibizumab, SD: Standard deviation, ERG: Electroretinography

To illustrate the changes in the visual system of RVO patients during ranibizumab treatment process, the information on mutual similarity (difference) of examined groups is presented in a graph plotted by discriminant analysis using Mahalanobis distance [Fig. 3].

Discussion

In our study, we simultaneously measured the concentrations of 27 cytokines by multiplex assay in aqueous humor of patients with macular edema and RVO. Eleven cytokines

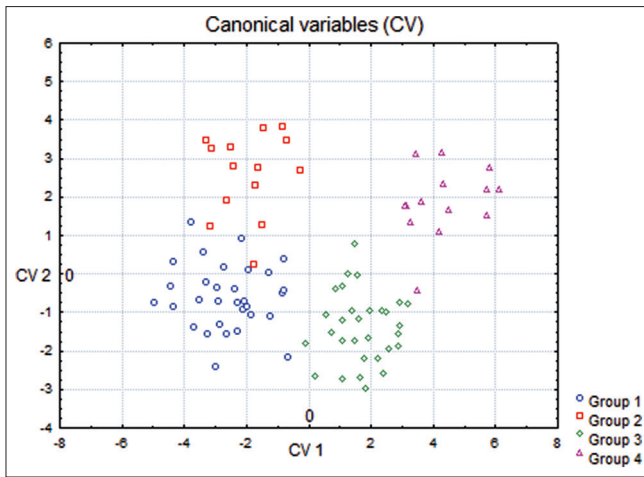


Figure 2: Dot graph for categorization of four groups in the system of coordinates depending on values of canonical variables

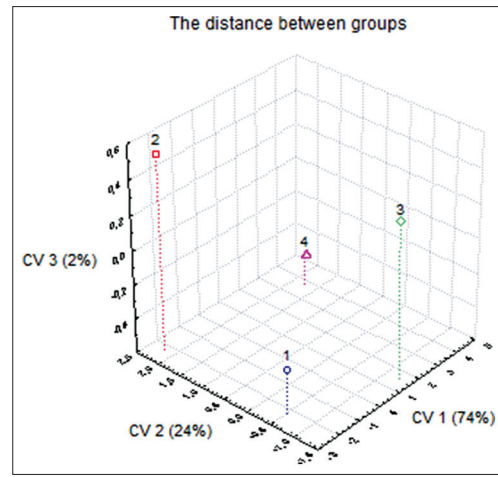


Figure 3: Graph of mutual similarity (difference) for examined groups in discriminant analysis

Table 4: Baseline cytokines concentrations in patients with “sufficient” and “insufficient” clinical response to IVR

Cytokine (pg/mL)	Group 1 (+) (n=30)	Group 2 (-) (n=14)	P _U
	1	2	
VEGF	1053.4±797.35	2140.01±2547.46	1-2<0.05
RAIL-1	25.32±23.84	19.34±19.84	
IL-6	104.67±194.43	120.01±88.59	
IL-8	73.09±77.03	277.09±372.26	1-2<0.05
IL-9	13.20±13.95	11.31±10.57	1-2<0.05
IL-10	14.11±10.17	25.50±18.31	1-2<0.05
IL-12p70	87.03±58.63	139.95±154.34	
IL-13	71.62±110.18	172.43±177.05	1-2<0.05
IL-15	4.34±3.48	5.68±3.24	
MCP-1	488.64±410.8	736.43±610.41	1-2<0.05
RANTES	14.19±26.94	7.58±8.45	

The data is presented as mean±SD. P - significance of differences in groups (U - Mann–Whitney U-test). IVR: Intravitreal ranibizumab, SD: Standard deviation, VEGF: Vascular endothelial growth factor, RAIL-1: Receptor antagonist of interleukin-1, IL: Interleukin, MCP-1: Monocyte chemoattractant protein-1, RANTES: Regulated on activation, normal T expressed and secreted

were found to be significantly increased compared to control group.

More than 100 types of different cytokines have been described by the current time.^[6] All cytokines are categorized in several groups (families) according to their biological activity, the most common of which are IFNs, TNFs, various families of interleukins, growth factors, and others.^[3] Cytokines are quite multifunctional: They induce, regulate, and limit inflammation, stimulate growth, proliferation and differentiation of cells as well as metabolism. Cytokines are universal, interchangeable, and pleiotropic, i.e., the same cytokines can interact with the receptors of different cell types, and similar-type cytokines can have opposite biological effects, whereas different type cytokines can have the same biological effect.^[3] However, despite this multifunctionality, particular mediators have certain prevailing features.

Particular groups of cytokines can be classified in the following simplified way:

- Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-12, IL-15, IFN-γ, TNF-α, etc.) activate T- and B-lymphocytes, and stimulate chemotaxis as well as phagocytic and cytotoxic activity. This group also includes chemotaxins (MCP-1, IL-8, and RANTES) responsible for traffic (chemotaxis) of immunocompetent cells to the area of inflammation
- Anti-inflammatory cytokines (IL-4, IL-10, IL-13, IL-17, RAIL-1, etc.) inhibit the inflammation and the synthesis of pro-inflammatory cytokines, stimulate proliferation of B-cells, synthesis of immunoglobulins, antibodies, promote finishing of acute-phase inflammation and generation of fibrosis
- Growth factors (VEGF, FGF, NGF, PDGF, IGF, GM-CSF, G-CSF, TGF, etc.) activate proliferation and differentiation of cells. They are involved in the process of apoptosis and angiogenesis and stimulate the “survivability” of cells.

The role of cytokines in the immunopathogenesis of various diseases has been already established. The discovery of how cytokine regulation of pathologies works prompted implementation of new methods of cytokine (substitutive) and anti-cytokine treatment in medical practice and ophthalmology is not an exception. The scientific interest in tissular and cellular regulators of the eye has grown quite recently. Thus, the first studies dedicated to cytokine concentration in uveitis were made in the early 1990s. Interesting data were received as a result of assessment of response after intraocular injection of particular inflammatory cytokines and growth factors in enucleated eyes of laboratory animals.^[7,8] A number of studies were dedicated to research of the inflammatory nature of diabetic retinopathy and diabetic macular edema, as well as to the role of VEGF and inflammatory cytokines in the pathogenesis of proliferative changes.^[9-11] Ophthalmologists started to treat patients with macular edema by intravitreal injections of corticosteroids (triamcinolone acetonide) supposing an inflammatory nature of macular edema.^[12,13] The authors noted the positive effect expressed in the reduction of macular edema. However, the effect lasted only for a short time, and there were adverse reactions secondary to steroid-induced glaucoma and cataract.

At present, there is increasing interest in the research of the intraocular level/balance of cytokines and for the discovery of cytokine-developing mechanisms of different eye disorders including RVO.

A specific breakthrough in the application of anti-cytokine strategy in modern ophthalmology was the development of anti-angiogenic agents (anti-VEGF antibodies), the effectiveness of which was showed in different kinds of vascular pathologies including RVO.^[1,14]

The role of VEGF in macular edema pathogenesis in RVO is quite well established. Today, it is clear that RVO patients are characterized by increased VEGF in their eyes, and the severity of macular edema directly correlates with VEGF and IL-6 levels.^[2,15] Similar correlations were observed among VEGF level, neovascularization of the iris, and vascular permeability in patients with ischemic RVO.^[16] Vascular overpermeability that caused macular edema was associated with hypersecretion of VEGF.^[1,17] Macrophages and monocytes have been described to secrete a wide range of pro-inflammatory mediators that are increasing vascular permeability. Moreover, IL-6, as one of the most important inducers of acute-phase proteins, can also stimulate hypersecretion of VEGF.^[2,18]

The more significant changes in the level of examined cytokines were noted in the sub-group of CRVO patients compared to patients with BRVO. The effectiveness of anti-angiogenic treatment was higher in BRVO patients than in CRVO patients which can probably be explained by a more severe retinal damage in CRVO.

Depression of ERG parameters and changing of visual functions and the extent of macular edema upon evaluation of initial clinical state of patients confirmed the depth of neuroretinal ischemic damage in fovea according to previously defined criteria of severity.^[19] Stage I (low) is characterized by a small zone of foveal nonperfusion (<1 quadrant), decrease of 50–60% in OPs (down to 30–20 mkV), and moderate decrease of 15–20% in b-wave amplitude (down to 110 mkV). Stage II (moderate) is characterized by a moderate zone of foveal nonperfusion (1–2 quadrants), decrease of 65–80% in OPs (down to 20–15 mkV), and moderate decrease of 20–30% in b-wave amplitude (down to 100 mkV). Stage III (high) is characterized by a massive zone of foveal nonperfusion (2 and more quadrants), evident decrease of 80% or more in OP (<15 mkV), and of 40% in b-wave amplitude (<100 mkV). As seen in Table 3, it is namely these parameters that allow us to discriminate the two groups – “sufficient” and “insufficient” clinical effect. Thus, all the patients with “insufficient” treatment effect were categorized with stages II and III retinal ischemia according to the classification provided.

Patients with “insufficient” response to IVR were characterized by altered concentrations of factors – markers of ischemia (VEGF), intensity of inflammation (IL-8, MCP-1), as well as factors that can be attributed to the failure of local inflammation limiting processes (IL-10, IL-13).

The results obtained in our study has shown that the predictors of “partial” response to single IVR include not only anatomical location of RVO (central vein or its branches), but also the stages of retinal ischemia, fovea involvement, and high concentrations of a number of cytokines (VEGF, IL-8,

MCP-1, IL-10, and IL-13). The obtained results could serve as a basis for further definition of the threshold for aqueous humor cytokines concentrations using receiver operating characteristic analysis which could potentially improve the accuracy of the prognostic model in predicting of anti-VEGF therapy efficacy.

The most important results were received from pooled analysis of all parameters in groups with “sufficient” and “insufficient” response to treatment in pre- and post-IVR periods (groups 1 and 2; 3 and 4). According to the analysis, the most significant criterion at baseline and after IVR was the OP index characterized the level of retinal ischemia. Finally, the high level of significance of Fisher criterion may indicate the reliability of the model.

Conclusions

The results of the study showed that RVO is accompanied by immuno-inflammatory processes with overexpression of VEGF and other pro- and anti-inflammatory cytokines and chemokines. The intensity of cytokine reactions is correlated with the extent of ischemic retinal damage. Intravitreal injection of ranibizumab is accompanied by a decrease in the level of VEGF and other cytokines/chemokines in aqueous humor. Defining the stage of retinal ischemia before the treatment could help to determine the prognosis of anti-VEGF therapy effectiveness and to assess the need for additional treatment.

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Conflicts of interest

There are no conflicts of interest.

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