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Systemic biomarkers of apoptosis and endothelial activation in the pathogenesis of rubeosis iridis and iridocorneal angle neovascularisation in secondary neovascular glaucoma

Abstract

Aim. To assess systemic levels of CD95 (Fas) and ICAM-1 (CD54) in patients with secondary neovascular glaucoma (NVG), to determine the relationship with the severity of iris rubeosis and the iridocorneal angle, and to evaluate the role of endothelial apoptosis in neovascular remodelling of the anterior segment of the eye.

Materials and methods. A single-centre cross-sectional study included 186 eyes with secondary NVG (96 associated with proliferative diabetic retinopathy and 90 following retinal vein occlusion) and 34 eyes in the control group. The severity of rubeosis was assessed using the Weiss grading scale. CD95 and ICAM-1 levels in peripheral blood were measured using an immunocytochemical method.

Results. In patients with NVG, CD95 and ICAM-1 levels were significantly higher compared with controls ($p < 0.001$). Both markers positively correlated with the severity of rubeosis according to Weiss (CD95: $rs = 0.46$; ICAM-1: $rs = 0.45$; $p < 0.001$) and with intraocular pressure. In a multivariable ordinal logistic regression model, CD95 and ICAM-1 remained independently associated with more severe rubeosis after adjustment for aetiology and intraocular pressure (CD95: OR = 2.31; 95% CI 1.27-4.18; $p = 0.006$; ICAM-1: OR = 2.48; 95% CI 1.56-3.95; $p < 0.001$). ROC analysis demonstrated moderate discriminative ability of the markers for detecting clinically significant rubeosis (Weiss ≥ II): AUC = 0.729 for CD95 and AUC = 0.730 for ICAM-1. Cut-off values were 687 cells/μL and 542 cells/μL, respectively.

Conclusions. CD95 (Fas) and ICAM-1 (CD54) are associated with the severity of rubeosis in secondary NVG and may serve as systemic biomarkers for neovascular remodelling and risk stratification of disease progression.

Keywords: CD95 (Fas); ICAM-1 (CD54); angiogenesis; neovascular remodelling; endothelial dysfunction; apoptosis; risk stratification.

INTRODUCTION

Secondary neovascular glaucoma is among the most severe forms of glaucomatous eye damage and remains one of the leading causes of irreversible vision loss in patients with ischaemic retinal diseases. The disease most commonly develops in association with proliferative diabetic retinopathy (PDR) and retinal vein occlusion (RVO). Both conditions are characterised by chronic tissue hypoxia, activation of angiogenesis and the formation of pathological newly formed vessels in the anterior and/or posterior segment of the eye. Rubeosis iridis and neovascularisation of the iridocorneal angle are key clinical manifestations of this process. These clinical signs determine further progression of ophthalmic hypertension and the development of refractory glaucoma. Despite the

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use of vascular endothelial growth factor (VEGF) inhibitors and modern laser technologies, the clinical course of the disease remains highly variable. Such variability suggests that additional pathophysiological mechanisms are involved and that the disease process extends beyond local angiogenesis alone.

In the latest review of NVG, S. Gerçeker Demircan & A. Şanal Doğan [1] pointed out that the clinical course of the disease is determined by a complex interaction between retinal ischaemia, pathological angiogenesis, inflammation and remodelling of the anterior segment of the eye. S. Senthil *et al*. [2] noted that progression of rubeosis iridis and neovascularisation of the anterior chamber angle depends not only on VEGF-mediated mechanisms. Concomitant activation of inflammatory and vascular signalling pathways also affects it, influencing disease severity and treatment outcomes. D. Urbonavičiūtė *et al*. [3] also showed that neovascular glaucoma has a multicomponent pathogenesis, in which ischaemia, angiogenesis, inflammation and fibrovascular remodelling form a single pathological cascade.

Particular attention has been paid to endothelial activation as one of the key mechanisms of neovascular remodelling. Analysing current views on the pathogenesis of RVO, M. Lendzioszek *et al*. [4] showed that ischaemic damage is accompanied by activation of pro-inflammatory signalling pathways, impaired endothelial homeostasis and remodelling of the vascular wall. The authors stressed that endothelial dysfunction is not only a consequence of retinal ischaemia. It can also actively contribute to further progression of the pathological process. J. Tang & T.S. Kern [5] noted that chronic retinal hypoxia stimulates the production of VEGF and other pro-angiogenic mediators. These mediators support the development of rubeosis iridis, neovascularisation of the iridocorneal angle and pathological vascular remodelling.

Intercellular adhesion molecule-1, ICAM-1 (CD54), is of particular interest in this context. It reflects endothelial activation and enhanced leukocyte-endothelial interaction. In a comprehensive review of vascular complications of diabetes mellitus, D.R. Yang *et al*. [6] showed that endothelial dysfunction is accompanied by increased expression of adhesion molecules, including ICAM-1, microvascular inflammation and impaired vascular homeostasis. The authors underlined that endothelial activation is an essential mechanism in the progression of vascular remodelling and tissue ischaemia. Similar findings were reported by S.F. Abcouwer [7], who showed that activation of pro-inflammatory cytokines and adhesion molecules is an important component of ischaemic retinal injury and pathological angiogenesis, contributing to chronic inflammation and vascular remodelling.

Apoptosis-associated mechanisms are also critical alongside endothelial activation. L. Hu *et al.* [8] showed that the CD95 (Fas) system is not only one of the key regulators of programmed cell death. It also participates in inflammation, intercellular signalling and tissue remodelling. The authors emphasised that Fas-mediated signalling pathways can influence the course of chronic vascular-inflammatory diseases through a combination of apoptotic and immunoregulatory effects. These data broaden the understanding of CD95. It may act both as a marker of cellular damage and a potential participant in pathological angiogenesis and neovascular tissue remodelling.

C. Mishra & J.J. Meyer [9], analysing the mechanisms of iris neovascularisation, concluded that the interaction of ischaemia, inflammation, endothelial activation and tissue remodelling forms the biological basis for rubeosis progression. The authors also outlined the need to determine systemic biomarkers capable of reflecting the activity of these processes. O.V. Guzun *et al.* [10] showed that elevated ICAM-1 levels were linked to a more severe course of diabetic NVG and a less favourable response to treatment. In another study, E. Doğan *et al*. [11] found increased systemic inflammatory biomarkers, including NLR, SII and TyG, in patients with neovascular glaucoma. These findings support further investigation of systemic biological mechanisms involved in the development and progression of the disease.

Despite growing evidence on the role of inflammation, endothelial dysfunction and apoptosis in ischaemic eye diseases, the relationship between systemic levels of CD95 (Fas), ICAM-1 (CD54) and the severity of rubeosis iridis and neovascularisation of the iridocorneal angle in secondary NVG remains insufficiently studied. Further research is needed to clarify systemic mechanisms of neovascular remodelling in the anterior segment of the eye and to identify biomarkers that can reflect the biological activity of rubeosis and disease severity. The purpose of the study was to assess systemic levels of CD95 (Fas) and ICAM-1 (CD54) in patients with secondary neovascular glaucoma associated with proliferative diabetic retinopathy and retinal vein occlusion. The framework of the above purpose encompassed defining the relationship with the severity of rubeosis iridis and the iridocorneal angle, and evaluating the possible role of systemic apoptosis-endothelial disturbances in neovascular remodelling of the anterior segment of the eye.

MATERIALS AND METHODS

Study design and ethical considerations. A single-centre cross-sectional observational study was conducted. It included 186 patients, corresponding to 186 eyes, with secondary neovascular glaucoma. The study was carried out at the State Institution "V.P. Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine" during 2018-2024. The study was performed in accordance with the World Medical Association Declaration of Helsinki [12] and the standards of the International

Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [13]. The Bioethics Committee of the State Institution "V.P. Filatov Institute of Eye Diseases and Tissue Therapy" (Protocol No. 4, 2024) approved it. All participants provided written informed consent. The data were anonymised in line with confidentiality requirements. All procedures remained within standard ophthalmological practice.

Participants and groups. The study researched the sample of 186 eyes with NVG: 96 with NVG related to proliferative diabetic retinopathy (NVG/PDR) and 90 with NVG following retinal vein occlusion (NVG/RVO). The control group consisted of 34 eyes from age-matched individuals without rubeosis iridis or glaucoma. The inclusion criteria were a confirmed diagnosis of NVG associated with PDR or RVO, the presence of rubeosis iridis, and uncontrolled intraocular pressure (IOP) ≥ 30 mmHg. The exclusion criteria were secondary NVG of another origin, severe systemic disease that could affect immune parameters, and the absence of clinical signs of anterior segment neovascularisation.

Clinical examination. All participants underwent a standard ophthalmological examination, including visual acuity testing, biomicroscopy, ophthalmoscopy and gonioscopy. IOP was measured by Goldmann applanation tonometry. The degree of iris neovascularisation was assessed using the classic Weiss grading scale (1971), which presupposes four stages. Stage I refers to scattered neovascular vessels located near the pupillary margin. Stage II describes neovascular proliferation extending to the mid-periphery of the iris. Stage III is characterised by a vascular network associated with anterior synechiae and incomplete angle closure. Stage IV indicates a dense neovascular network with anterior synechiae and complete closure of the iridocorneal angle [14]. No patients with Weiss stage IV were present in the study cohort. Weiss ≥ II was used as a quantitative measure of clinically significant rubeosis.

Biomarker assessment. Expression of CD95 (Fas) and ICAM-1 (CD54) on lymphocytes was determined in peripheral venous blood samples by indirect immunofluorescence using monoclonal antibodies against the corresponding antigens. The R.E. Kavetsky Institute of Experimental Pathology, Oncology, and Radiobiology of the National Academy of Sciences of Ukraine, Kyiv, Ukraine produces the antibodies. Fluorescein isothiocyanate (FITC) from the same manufacturer was used to visualise the immunological reaction. A lymphocyte suspension was obtained by centrifuging peripheral blood on a Ficoll density gradient (1.076 g/cm^3; Simesta, Ukraine), followed by two rounds of cell washing. After smear preparation and fixation, the samples were incubated with specific monoclonal antibodies, washed, and then incubated with FITC for 3 hours. After the final wash, the slides were examined using an iSCOPE fluorescence microscope (Euromex, the Netherlands). During the analysis, the proportion of positively stained cells among 100 lymphocytes was defined. To standardise the results, the obtained values were recalculated as the absolute number of CD95$^+$ and CD54$^+$ cells per 1 μL of peripheral blood, taking into account the absolute lymphocyte count in the complete blood count. All analyses were performed in the same laboratory according to a unified protocol, using the same reagents and analytical conditions [15].

Endpoints. The primary endpoint was the severity of rubeosis iridis and neovascularisation of the iridocorneal angle according to the Weiss grading scale, considered as an ordinal clinical characteristic of neovascular process activity. The secondary endpoint was the presence of clinically significant rubeosis (Weiss ≥ II). It was employed for ROC analysis and stratification of neovascular process activity. Clinical characteristics and biomarker levels in patients with neovascular glaucoma and in the control group are presented in Table 1.

Table 1. Clinical characteristics and biomarker levels in patients with neovascular glaucoma and in the control group

Parameters	NVG/PDR (n = 96)	NVG/RVO (n = 90)	Control (n = 34)
	Me (Q1-Q3), n (%)		
Male/female	42 (44%) / 54 (56%)	40 (44%) / 50 (56%)	13 (38%) / 21 (62%)
Age, years	66 (62-68.5) * ‡	63 (61-66)	63.5 (60-66)
Intraocular pressure, mmHg	36 (33-40) *	34 (33-40) *	15 (14-17)
Rubeosis of the iris (Weiss)			
⬡ I degree	50 (52%)	52 (58%)	–
⬡ II degree	28 (29%)	29 (32%)	–
⬡ III degree	18 (19%)	9 (10%)	–
CD95 (Fas), cells/μL	653 (610-832) *	612 (553-691) *	263.5 (220-283)
ICAM-1 (CD54), cells/μL	373 (345-566) *	368 (339-542) *	190.5 (186-197)

Table 1. Continued

Parameters	NVG/PDR (n = 96)	NVG/RVO (n = 90)	Control (n = 34)
	Me (Q1-Q3), n (%)		
Duration of diabetes/ Duration of retinal vein occlusion, months	132 (84-168) * ‡	15 (12-18) *	–
Cardiovascular diseases	56 (58%) *	53 (59%) *	15 (44%)

Note: data are presented as median (interquartile range) or number (%). * – $p < 0.05$ between control and PDR/RVO group; ‡ – $p < 0.05$ between NVG/PDR and NVG/RVO group at the corresponding Weiss stage. NVG – neovascular glaucoma; PDR – proliferative diabetic retinopathy; RVO – retinal vein occlusion; ICAM-1 – intercellular adhesion molecule-1. CD95 and ICAM-1 levels are presented as the absolute number of positive lymphocytes per microlitre of peripheral blood
Source: developed by the authors

Statistical processing was Statistical analysis was performed using JASP software, version 0.19.2, and Python. Normal distribution of the biomarkers was rejected by the Shapiro-Wilk test (CD95: $p < 0.001$; ICAM-1: $p < 0.001$). This justified the use of non-parametric methods: the Mann-Whitney test for between-group comparisons, the Kruskal-Wallis test for multiple-group comparisons, and Spearman's coefficient (r_s) for correlations. Quantitative variables were presented as the median and interquartile range (Q1-Q3). To ensure statistical independence of observations, only one eye from each patient was included in the analysis. In cases of bilateral involvement, the eye with more pronounced manifestations of rubeosis iridis and neovascularisation of the iridocorneal angle according to the Weiss grading scale was selected.

Multivariable regression analysis was performed to identify independent predictors of rubeosis severity, with adjustment for NVG aetiology (PDR/RVO) and IOP. The diagnostic ability of CD95 and ICAM-1 for clinically significant rubeosis (Weiss ≥ II) was assessed using ROC analysis, with calculation of the area under the curve (AUC), the optimal cut-off value according to the Youden index, sensitivity, and specificity. Quartile analysis was performed to define dose-dependent effects, with the frequency of Weiss ≥ II calculated in each quartile. Values of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

CD95 and ICAM-1 levels in patients with NVG. Given the presumed role of apoptosis-associated and endothelial-inflammatory mechanisms in the development of the neovascular process, systemic levels of CD95 (Fas) and ICAM-1 (CD54) were assessed. Patients with NVG had significantly higher CD95 and ICAM-1 levels compared with controls ($p < 0.001$). The highest CD95 levels were observed in the NVG/PDR group. This may be an indication of a more pronounced activation of apoptosis-associated mechanisms in diabetic ischaemic injury. ICAM-1 levels were also higher in the NVG groups compared with controls, reflecting activation of systemic endothelial inflammation.

Association between biomarkers and the severity of rubeosis. In the overall NVG cohort, significant positive correlations were found between the severity of rubeosis according to Weiss and biomarker levels: $r_s = 0.46$ for CD95 and $r_s = 0.45$ for ICAM-1 (both $p < 0.001$). Significant associations with IOP were also identified: $r_s = 0.58$ for CD95 and $r_s = 0.61$ for ICAM-1 ($p < 0.001$).

Multivariable analysis. In the multivariable ordinal logistic regression model, increased CD95 and ICAM-1 levels were independently associated with more severe rubeosis iridis. The strongest association was observed for ICAM-1 (OR = 2.48; 95% CI 1.56-3.95; $p < 0.001$) (Table 2). This finding supports the pivotal role of endothelial activation and vascular inflammation in the development of neovascular remodelling of the anterior segment of the eye.

Table 2. Multivariate ordinal logistic regression analysis of the association between CD95, ICAM-1 and clinically significant rubeosis (Weiss ≥ II) in secondary NVG

Indicator	β	SE	OR (95% CI)	Wald χ^2	p
CD95	0.84	0.31	2. 31 (1.27-4.18)	7.41	0.006
ICAM-1 (CD54)	0.91	0.24	2. 48 (1.56-3.95)	14.36	<0.001
IOP	0.07	0.03	1.07 (1.01-1.14)	5.12	0.024
NVG/PDR (vs. NVG/RVO)	0.42	0.28	1.52 (0.88-2.64)	2.21	0.137
Cardiovascular pathology	0.29	0.25	1.34 (0.82-2.20)	1.37	0.241

Note: dependent variable: degree of rubeosis according to Weiss; OR – odds ratio; CI – confidence interval; NVG – neovascular glaucoma; IOP – intraocular pressure; PDR – proliferative diabetic retinopathy; RVO – retinal vein occlusion
Source: developed by the authors

Elevated CD95 was also associated with a higher likelihood of more severe rubeosis (OR = 2.31; 95% CI 1.27-4.18; $p = 0.006$). This reflects the involvement of apoptosis-associated signalling in the progression of ischaemic-reparative processes. After adjustment for IOP, NVG aetiology and concomitant cardiovascular

disease, the aetiological factor (PDR/RVO) lost statistical significance. This may suggest that systemic biological activity of the process plays a greater role than the nosological background of the disease.

Stratification of clinically significant rubeosis. ROC demonstrated comparable discriminative ability of CD95 (Fas) and ICAM-1 (CD54) for detecting clinically significant rubeosis (Weiss ≥ II) in patients with secondary neovascular glaucoma (Fig. 1).

Figure 1 shows that the areas under the ROC curves were 0.729 for CD95 and 0.730 for ICAM-1. These values correspond to moderate prognostic accuracy for both markers. Similar AUC values indicate a comparable contribution of apoptosis-associated and endothelial-inflammatory mechanisms to the progression of neovascular changes in the anterior segment of the eye. A non-parametric Kruskal-Wallis rank analysis was additionally performed to assess the association between biomarker levels and rubeosis grades according to Weiss in the NVG cohort (n = 186). For CD95, highly significant between-group differences were found across rubeosis grades ($H(2) = 43.149$; $p < 0.001$). Mean ranks increased consistently from Weiss I (n = 102; mean rank 74.26) to Weiss II (n = 57; 101.57) and Weiss III (n = 27; 149.13). This pattern reflects a monotonic increase in CD95 levels with greater severity of neovascular changes. A similar gradient pattern was observed for ICAM-1 (CD54): $H(2) = 42.30$; $p < 0.001$. Mean ranks were 74.20 for Weiss I (n = 102), 102.23 for Weiss II (n = 57) and 147.98 for Weiss III (n = 27). These results confirm the dose-dependent nature of the association between CD95, ICAM-1 and the degree of rubeosis. The findings are also consistent with the regression analysis and further emphasise the pathogenetic significance of both markers in the formation of active anterior segment neovascularisation. Biomarker levels progressively increased with greater severity of rubeosis iridis (p-trend < 0.001).

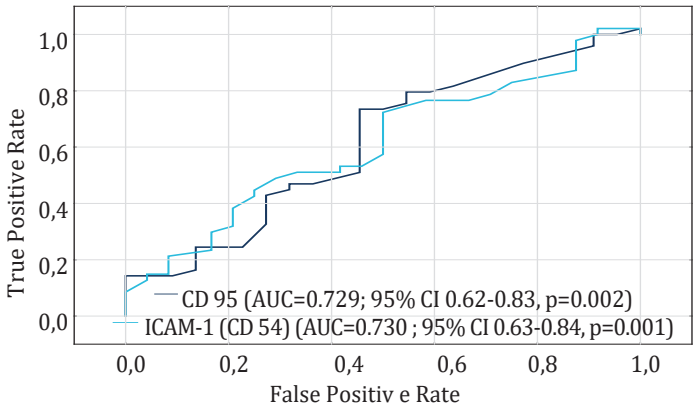

Figure 1. ROC curves for differentiating clinically significant rubeosis (Weiss ≥ II)

Note: receiver operating characteristic (ROC) curves showing the diagnostic performance of CD95 (Fas) and ICAM-1 (CD54) for detecting clinically significant rubeosis iridis (Weiss ≥ II) in patients with neovascular glaucoma. The diagonal dashed line indicates the random classification level

Source: developed by the authors

For stratification of clinically significant rubeosis (Weiss ≥ II), ROC analysis was performed with calculation of optimal cut-off values according to the Youden index. For CD95, the optimal cut-off value was 687 cells/μL. This corresponded to a sensitivity of 63.1% and a specificity of 83.3%, with an AUC of 0.729. For ICAM-1 (CD54), the optimal cut-off value was 542 cells/μL, with a sensitivity of 59.5% and a specificity of 85.3% at an AUC of 0.730. These values confirm the clinical applicability of both markers for quantitative identification of clinically significant rubeosis (Table 3).

Table 1. Optimal threshold values (Youden index) and diagnostic efficiency of CD95 and ICAM-1

Biomarker	AUC (95% CI)	Optimal threshold (Youden)	Sensitivity, %	Specificity, %
CD95 (Fas)	0.729 (0.62-0.83)	687.0	63.1	83.3
ICAM-1 (CD54)	0.730 (0.63-0.84)	542.0	59.5	85.3

Note: the values are obtained from the results of ROC analysis for the differentiation of clinically significant rubeosis of the iris (Weiss ≥ II). The optimal threshold values were determined by the Youden index (J = sensitivity + specificity − 1). AUC – area under the ROC curve

Source: developed by the authors

Quartile analysis showed a dose-dependent increase in the frequency of severe rubeosis iridis with rising CD95 and ICAM-1 levels. The proportion of patients with Weiss II-III rubeosis increased from 12.5%

in the lowest CD95 quartile to 71.2% in the highest quartile. A similar trend was observed for ICAM-1, with an increase from 15.1% to 74.5%, respectively. These findings display a graded increase in neovascular activity as systemic apoptosis-endothelial signalling becomes more pronounced (Fig. 2).

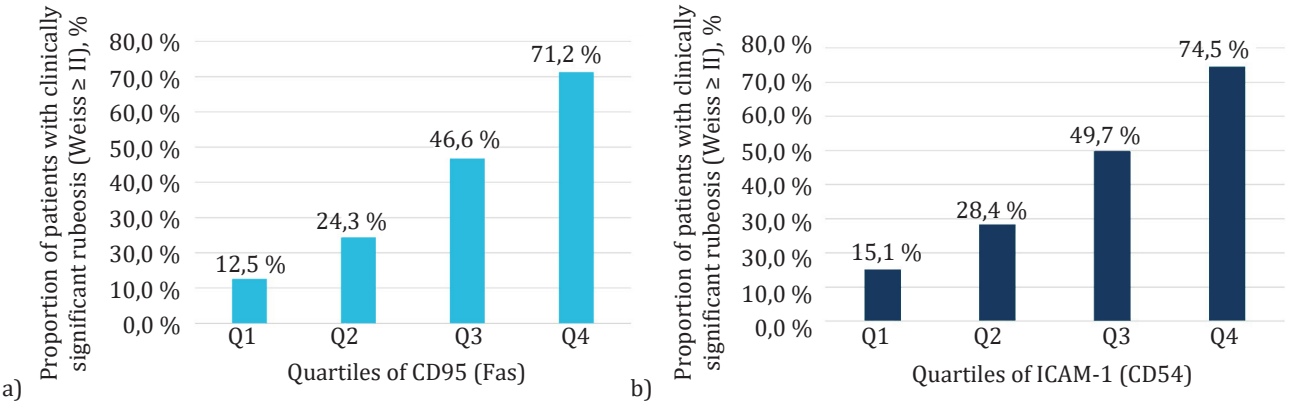

Figure 2. Frequency of clinically significant Weiss rubeosis ≥ II depending on the quartiles of CD95 (a) and ICAM-1 (CD54) (b) levels

Note: Q1 is the lowest quartile (25% of patients with the lowest marker values); Q4 is the highest quartile (25% of patients with the highest marker values). A statistically significant dose-dependent trend was found for both biomarkers (p-trend < 0.001)

Source: developed by the authors

Quartile analysis demonstrated a clear dose-dependent relationship between CD95 (Fas), ICAM-1 (CD54) levels and the frequency of clinically significant rubeosis (Weiss ≥ II) (Fig. 2). As the values increased from the lowest quartile (Q1) to the highest quartile (Q4), the frequency of pronounced neovascular changes in the anterior segment of the eye rose steadily for both biomarkers. The highest rates of rubeosis were recorded in patients whose CD95 and ICAM-1 levels corresponded to the fourth quartile. In contrast, clinically significant rubeosis was least frequent in the groups with the lowest marker concentrations. The statistically significant trend (p-trend < 0.001) validates a graded association between increasing systemic apoptosis-endothelial activation and progression of the neovascular process. These findings suggest that elevated CD95 and ICAM-1 levels are correlated with higher biological activity of rubeosis iridis and iridocorneal angle neovascularisation. The above findings may also reflect increasing pathological neovascular remodelling in secondary NVG.

The results prove a major role of systemic apoptosis-endothelial activation in the development and progression of rubeosis iridis and iridocorneal angle neovascularisation in secondary neovascular glaucoma. NVG is known to result from a complex interaction between retinal ischaemia, pathological angiogenesis, inflammation and remodelling of anterior segment tissues [1, 2]. Chronic retinal hypoxia stimulates the production of VEGF and other pro-angiogenic mediators. These mediators support the development of rubeosis iridis and neovascularisation of the anterior chamber angle [3, 5]. Current views on NVG pathogenesis, however, extend beyond VEGF-dependent mechanisms alone. The above views also involve endothelial dysfunction, systemic inflammation and immune activation [4].

In the present study, systemic markers of endothelial activation (ICAM-1/CD54) and apoptosis (CD95/Fas) were significantly elevated in patients with secondary NVG. In addition to it, these markers were also independently related to the severity of rubeosis. After adjustment for intraocular pressure and disease aetiology, both indicators retained a statistically significant association with the severity of neovascular changes. These findings presume that defining the biological activity of the process comprises local haemodynamic changes or the level of ophthalmic tone. Systemic mechanisms of vascular and cellular remodelling also appear to play a pivotal role.

The findings are consistent with the modern concept of NVG as a multifactorial vascular-inflammatory disease. In this model, anterior segment neovascularisation reflects an integrated ischaemic-inflammatory cascade [2, 3]. S. Gerçeker Demircan & A. Şanal Doğan [1] emphasised that the clinical course of NVG is determined by the degree of retinal ischaemia. Furthermore, it is shaped by the intensity of the inflammatory response, vascular remodelling and endothelial dysfunction. Similarly, M. Lendzioszek *et al.* [4] considered endothelial dysfunction one of the key mechanisms supporting the ischaemic-neovascular process in retinal vascular diseases.

ICAM-1 (CD54) deserves particular attention as a marker of endothelial activation and intercellular adhesion. D.R. Yang *et al.* [6] showed that in diabetic microangiopathy, endothelial dysfunction is accompanied by increased ICAM-1 expression, microvascular inflammation and impaired vascular homeostasis. S.F. Abcouwer [7]

also highlighted the important role of adhesion molecules in maintaining ischaemic inflammation and pathological angiogenesis. The present results support these observations, since the independent association between ICAM-1 and the severity of rubeosis persisted even after intraocular pressure had been taken into account. Additional support comes from the data of K. Siddiqui *et al*. [16] and the meta-analysis by Y. Yao *et al*. [17], which underscored systemic elevation of ICAM-1 levels in diabetic retinopathy and other ischaemic retinal diseases. The present findings are also consistent with previous data from the authors, who showed an association between elevated ICAM-1 levels, a more severe clinical course of diabetic neovascular glaucoma and a less favourable response to treatment [10]. In summary, these observations suggest that endothelial activation is not only a marker of ischaemic injury. It may also be a potential component of neovascular process progression.

The results also align well with data on the local cytokine microenvironment in NVG. S. Ohira *et al*. [18] demonstrated a marked increase in IL-6, IL-8, CCL2, TNF-α and VEGF levels in the aqueous humour of eyes with NVG. This increase partially persisted even after anti-VEGF therapy. T. Kokubun *et al.* [19] identified characteristic profiles of pro-inflammatory cytokines in patients with different forms of glaucoma, including NVG. These data highlight that impaired aqueous humour outflow in NVG should not be regarded only as a mechanical consequence of neovascularisation. It can also reflect persistent inflammatory-endothelial remodelling of anterior segment tissues.

Alongside endothelial activation, apoptosis-associated mechanisms play an important role in the formation of the neovascular phenotype. K. Seyrek *et al*. [20] and L. Hu *et al*. [8] showed that the CD95/Fas system is a multifunctional signalling platform. It regulates programmed cell death, inflammation, cell survival and tissue remodelling. Under conditions of chronic tissue injury, CD95 activation may involve non-apoptotic signalling pathways, including NF-κB, MAPK and PI3K/Akt. These pathways support a pro-inflammatory microenvironment and pathological remodelling. The elevated systemic CD95 levels found in patients with more severe rubeosis are consistent with these concepts. The involvement of Fas-mediated signalling in maintaining the neovascular process can be substantiated.

M. Lendzioszek *et al*. [4] noted that ischaemic retinal diseases are accompanied by a complex interaction between hypoxia, inflammation, endothelial dysfunction and vascular remodelling. The authors emphasised that progression of retinal ischaemia is related to activation of pro-inflammatory and pro-angiogenic signalling pathways. Such pathways may maintain the neovascular process even after the primary vascular injury has been eliminated. The present findings coincide with these views and submit a possible contribution of systemic mechanisms to the maintenance of pathological angiogenesis in secondary NVG. The role of systemic inflammation is supported by the findings of E.W. Böhm *et al*. [21]. The authors established an association between elevated systemic inflammatory indicators and the severity of clinical manifestations of retinal vein occlusion, as well as the impact on functional disease outcomes. Higher ICAM-1 and CD95 levels in NVG linked to proliferative diabetic retinopathy, compared with post-occlusive NVG, may reflect the chronic systemic nature of diabetic microangiopathy. Prolonged endothelial activation and inflammation accompany this process [6, 10]. Similar conclusions were reported by M. Kozman *et al*. [22], who showed that the risk of NVG in proliferative diabetic retinopathy is determined by local retinal ischaemia. Meanwhile, systemic metabolic factors, including poor glycaemic control and concomitant manifestations of diabetic microangiopathy, also become prerequisites for this risk. In retinal vein occlusion, by contrast, acute ischaemic and VEGF-mediated mechanisms appear to dominate to a greater extent [5].

The dose-dependent association of CD95 and ICAM-1 with the severity of rubeosis confirms the systemic nature of the pathological processes underlying neovascular remodelling of the anterior segment of the eye in secondary NVG. Given this, the findings support the inclusion of apoptosis and endothelial activation markers in the comprehensive assessment of patients. The use of the results may improve the objectivity of disease course prediction and help justify personalised treatment strategies.

CONCLUSIONS

1. Patients with secondary NVG had significantly elevated systemic levels of CD95 (Fas) and ICAM-1 (CD54) compared with the control group ($p < 0.001$). This makes activation of apoptosis-associated and endothelial-inflammatory mechanisms in neovascular remodelling of the anterior segment of the eye evident. The study also found a significant positive association between both biomarker levels and the severity of rubeosis iridis and iridocorneal angle neovascularisation according to Weiss. Significant associations with intraocular pressure were also observed, confirming the relationship with neovascular process activity.

2. In the multivariable ordinal logistic regression analysis, CD95 and ICAM-1 remained independent predictors of more severe rubeosis after adjustment for disease aetiology and intraocular pressure (CD95: OR = 2.31; 95% CI 1.27-4.18; $p = 0.006$; ICAM-1: OR = 2.48; 95% CI 1.56-3.95; $p < 0.001$). This specifies an independent contribution of apoptosis-endothelial mechanisms to the progression of neovascular changes. ROC analysis demonstrated moderate discriminative ability of both markers for detecting clinically significant rubeosis (AUC = 0.729 for CD95 and AUC = 0.730 for ICAM-1). The cut-off values of 687 cells/μL and 542 cells/μL, respectively, may be used for risk stratification of a more severe disease course.

3. Quartile analysis revealed a statistically significant dose-dependent association between increasing

CD95 and ICAM-1 levels and the frequency of clinically significant rubeosis (p-trend < 0.001). This further supports the role of systemic apoptosis-endothelial activation in the pathogenesis of secondary NVG. The findings suggest that CD95 and ICAM-1 may serve as additional biomarkers for assessing the biological activity of rubeosis, objectifying the risk of disease progression and improving personalised approaches to patient management.

4. A promising direction for later research is to study the prognostic value of these biomarkers during long-term follow-up and to integrate aforementioned biomarkers into multivariable models for predicting the course and treatment outcomes of secondary NVG.

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CONFLICT OF INTEREST

None.

REFERENCES

[1] Gerçeker Demircan S, Şanal Doğan A. Neovascular glaucoma: Comprehensive evaluation of etiology, treatment modalities, and visual prognosis. BMC Ophthalmol. 2025;25:540. DOI: 10.1186/s12886-025-04389-x

[2] Senthil S, Dada T, Das T, Kaushik S, Puthuran GV, Philip R, et al. Neovascular glaucoma – a review. Indian J Ophthalmol. 2021;69(3):525–34. DOI: 10.4103/ijo.ijo_1591_20

[3] Urbonavičiūtė D, Buteikienė D, Janulevičienė I. A review of neovascular glaucoma: Etiology, pathogenesis, diagnosis, and treatment. Medicina. 2022;58(12):1870. DOI: 10.3390/medicina58121870

[4] Lendzioszek M, Bryl A, Poppe E, Zorena K, Mrugacz M. Retinal vein occlusion-background knowledge and foreground knowledge prospects – a review. J Clin Med. 2024;13(13):3950. DOI: 10.3390/jcm13133950

[5] Tang J, Kern TS. Inflammation in diabetic retinopathy. Prog Retin Eye Res. 2011;30(5):343–58. DOI: 10.1016/j.preteyeres.2011.05.002

[6] Yang DR, Wang MY, Zhang CL, Wang Y. Endothelial dysfunction in vascular complications of diabetes: A comprehensive review of mechanisms and implications. Front Endocrinol. 2024;15:1359255. DOI: 10.3389/fendo.2024.1359255

[7] Abcouwer SF. Angiogenic factors and cytokines in diabetic retinopathy. J Clin Cell Immunol. 2013;1(11):1–12. DOI: 10.4172/2155-9899

[8] Hu L, Lu J, Fan H, Niu C, Han Y, Caiyin Q, et al. FAS mediates apoptosis, inflammation, and treatment of pathogen infection. Front Cell Infect Microbiol. 2025;15:1561102. DOI: 10.3389/fcimb.2025.1561102

[9] Mishra C, Meyer JJ. Neovascular glaucoma. Treasure Island (FL): StatPearls Publishing; 2022.

[10] Guzun OV, Zadorozhnyy OS, Velychko LM, Bogdanova OV, Dumbrăveanu LG, Cuşnir VV, et al. The effect of the intercellular adhesion molecule-1 and glycated haemoglobin on the management of diabetic neovascular glaucoma. Rom J Ophthalmol. 2024;68(2):135–42. DOI: 10.22336/rjo.2024.25

[11] Doğan E, Aydemir S, Özata Gündoğdu K, Alagöz G. Assessment of systemic inflammatory biomarkers in neovascular glaucoma. Ocul Immunol Inflamm. 2025;33(9):2022–8. DOI: 10.1080/09273948.2025.2547909

[12] World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4. DOI: 10.1001/jama.2013.281053

[13] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Harmonised Guideline E6(R3): Guideline for Good Clinical Practice. Geneva: ICH; 2025. 86 P.

[14] Teich SA, Walsh JB. A grading system for iris neovascularization. Prognostic implications for treatment. Ophthalmology. 1981;88(11):1102–6. DOI: 10.1016/s0161-6420(81)34900-8

[15] Velychko LM, Bogdanova OV. A method for assessing the receptor-modifying effect of pharmacological immunotropic drugs on markers of cell activation. Patent of Ukraine № 103483. 2015: 24.

[16] Siddiqui K, George TP, Mujammami M, Isnani A, Alfadda AA. The association of cell adhesion molecules and selectins (VCAM-1, ICAM-1, E-selectin, L-selectin, and P-selectin) with microvascular complications in patients with type 2 diabetes: A follow-up study. Front Endocrinol. 2023;14:1072288. DOI: 10.3389/fendo.2023.1072288

[17] Yao Y, Du J, Li R, Zhao L, Luo N, Zhai JY, et al. Association between ICAM-1 level and diabetic retinopathy: A review and meta-analysis. Postgrad Med J. 2019;95(1121):162–8. DOI: 10.1136/postgradmedj-2018-136102
[18] Ohira S, Inoue T, Shobayashi K, Iwao K, Fukushima M, Tanihara H. Simultaneous increase in multiple proinflammatory cytokines in the aqueous humor in neovascular glaucoma with and without intravitreal bevacizumab injection. Invest Ophthalmol Vis Sci. 2015;56(6):3541–8. DOI: 10.1167/iovs.14-15918
[19] Kokubun T, Tsuda S, Kunikata H, Yasuda M, Himori N, Kunimatsu-Sanuki S, et al. Characteristic profiles of inflammatory cytokines in the aqueous humor of glaucomatous eyes. Ocul Immunol Inflamm. 2018;26(8):1177–88. DOI: 10.1080/09273948.2017.1327605
[20] Seyrek K, Espe J, Reiss E, Lavrik IN. The crosstalk of apoptotic and non-apoptotic signaling in CD95 system. Cells. 2024; 13(21):1814. DOI: 10.3390/cells13211814
[21] Böhm EW, Wolfrum P, Welzel AM, Schuster AK, Wagner FM, Gericke A, et al. Systemic inflammation in retinal vein occlusion and its role in clinical outcomes of secondary macular edema. J Clin Med. 2026;15(2):407. DOI: 10.3390/jcm15020407
[22] Kozman M, Nyalakonda R, Farson L, Hunt M, Chuang A, Movahedan A, et al. Risk factors associated with neovascular glaucoma in eyes with proliferative diabetic retinopathy. Invest Ophthalmol Vis Sci. 2025;66(8):1660.

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Системні біомаркери апоптозу та ендотеліальної активації у патогенезі рубеозу райдужки та іридокорнеального кута при вторинній неоваскулярній глаукомі

Анотація

Мета. Оцінити системні рівні CD95 (Fas) та ICAM-1 (CD54) у пацієнтів із вторинною неоваскулярною глаукомою (НВГ), визначити їх зв'язок із тяжкістю рубеозу райдужки та іридокорнеального кута, а також оцінити роль апоптоз-ендотеліальних порушень у неоваскулярному ремоделюванні переднього сегмента ока.

Матеріали та методи. В одноцентрове перехресне дослідження включено 186 очей із вторинною НВГ (96 – на тлі проліферативної діабетичної ретинопатії та 90 – після оклюзії вен сітківки) і 34 ока контрольної групи. Тяжкість рубеозу оцінювали за шкалою Weiss. Рівні CD95 та ICAM-1 у периферичній крові визначали імуноцитохімічним методом.

Результати дослідження. У пацієнтів із НВГ рівні CD95 та ICAM-1 були достовірно вищими порівняно з контролем ($p < 0,001$). Обидва маркери позитивно корелювали з тяжкістю рубеозу за Weiss (CD95: $rs = 0,46$; ICAM-1: $rs = 0,45$; $p < 0,001$) та рівнем внутрішньоочного тиску. У багатофакторній ordinal logistic regression моделі CD95 та ICAM-1 залишалися незалежно асоційованими з більш тяжким рубеозом після корекції на етіологію та внутрішньоочний тиск (CD95: OR = 2,31; 95 % CI 1,27-4,18; $p = 0,006$; ICAM-1: OR = 2,48; 95 % CI 1,56-3,95; $p < 0,001$). ROC-аналіз показав помірну дискримінаційну здатність маркерів щодо виявлення клінічно значущого рубеозу (Weiss ≥ II): AUC = 0,729 для CD95 та AUC = 0,730 для ICAM-1. Порогові значення становили 687 кл/мкл та 542 кл/мкл відповідно.

Висновок. CD95 (Fas) та ICAM-1 (CD54) асоціюються з тяжкістю рубеозу при вторинній НВГ і можуть розглядатися як системні біомаркери неоваскулярного ремоделювання та стратифікації ризику прогресування захворювання.

Ключові слова: CD95 (Fas); ICAM-1 (CD54); ангіогенез; неоваскулярне ремоделювання; ендотеліальна дисфункція; апоптоз; стратифікація ризику