ORIGINAL ARTICLE

CONTENTS

Interleukins (IL-1β, IL-4, IL-6, and IL-8) in aqueous humor as potential biomarkers of diabetic retinopathy severity

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ABSTRACT

Aims: To determine the concentrations of interleukins (IL1β, IL4, IL6, IL8) in the aqueous humor and their association with diabetic retinopathy severity.

Materials and Methods: Singlecenter crosssectional study with prospective sampling of 110 adults with type 2 diabetes mellitus spanning five categories of diabetic retinopathy and 25 nondiabetic controls. Aqueous humor (0.1 mL) obtained during phacoemulsification was analyzed by ELISA. Nonparametric tests assessed group differences; optimal thresholds were derived by multiclass onevsall ROC-analysis, followed by clinically relevant binary stratification (mild/moderate vs severe DR). Spearman rank correlations evaluated relationships with age, diabetes duration, fasting glucose, HbA1c, and Cpeptide.

Results: All interleukins differed between groups ($p < 0.001$). IL1β and IL6 increased with stage; IL8 peaked at severe nonproliferative disease and declined in proliferative disease; IL4 decreased. Multiclass accuracies were modest: 56%, 58%, 53%, and 44% for IL-6, IL8, IL1β, and IL4. In the binary model, IL6 achieved 72.6% accuracy (95% CI 64.3-79.9), with cutoffs of 34.4-86.2 and >86.2 pg/mL for mild/moderate and severe disease. IL8 was supportive (62.2%; severe >216.9 pg/mL). IL1β and IL4 each yielded about 55.6%. Correlations were weak to moderate: IL6 and IL8 tracked diabetes duration and glycemia, while IL4 inversely tracked Cpeptide.

Conclusions: IL6 is the most informative single local biomarker for binary severity stratification of diabetic retinopathy, with IL8 as an adjunct and IL1β/ IL4 of limited standalone value. IL6 thresholds may guide riskadapted followup and monitoring with optical coherence tomography and optical coherence tomography angiography. External validation, preanalytical standardization, and prospective outcome studies are needed.

KEY WORDS: diabetic retinopathy, diabetes mellitus type 2, aqueous humor, interleukins, interleukin-1β, interleukin-4, interleukin-6, interleukin-8, biomarkers, disease severity

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INTRODUCTION

Diabetic retinopathy (DR) remains one of the leading causes of vision loss in the workingage population, and its burden is rising in parallel with the global epidemic of type 2 diabetes mellitus (T2DM) [1]. According to the 11th edition of the International Diabetes Federation (IDF) Atlas, 588.7 million adults (20–79 years; prevalence 11.1%) were living with diabetes in 2024, with numbers projected to reach 852.5 million (13.0%) by 2050 – trends that will inevitably amplify the incidence of DR [1]. In the global landscape of vision loss, DR consistently ranks among the principal causes of blindness and moderatetosevere visual impairment in individuals aged ≥50 years, as documented by the GBD/VLEG consortium analysis of 1990-2020 data [2]. In clinical practice, disease severity is graded using the International Clinical Diabetic Retinopathy (ICDR, 2003) scale, which distinguishes DR0, mild/moderate/severe nonproliferative DR (NPDR), and proliferative DR (PDR), whereas diabetic macular edema (DME) is evaluated separately [3]. However, this discrete, structurebased grading does not fully capture early ischemic and neuroinflammatory processes that precede overt microvascular signs [4, 5].

Optical coherence tomography angiography (OCTA) has enabled the visualization of retinal perfusion defects – particularly within the deep capillary plexus (DCP) – that are associated with downstream complications as early as moderate NPDR and even in individuals with T2DM without clinically apparent DR [4, 5]. This mismatch between the continuous pathophysiology of DR progression and categorical clinical grading underscores the need for local biomarkers capable of biochemically profiling the retinal microenvironment and complementing imaging data to refine risk stratification for the development and progression of DR.

Chronic lowgrade inflammation is recognized as a principal driver of DR pathogenesis: hyperglycemia activates proinflammatory cells, augments oxidative stress,

and impairs endothelial function, thereby triggering a cascade of mediators that damage the retinal neurovascular unit [6, 7]. A central role in this cascade is played by the NLRP3 inflammasome (NODlike receptor family, pyrin domaincontaining 3): its activation induces secretion of IL1β/IL18 and pyroptotic cell death, amplifying inflammation and microvascular dysfunction [8]. Against this background, interleukins – as universal regulators of innate and adaptive immunity – form inflammatory "signatures" that can be locally monitored in DR, given their amenability to qualitative and quantitative assessment in ocular tissues.

IL1β is a classical proinflammatory mediator that injures the retinal capillary endothelium via NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation, increased oxidative stress, and mitochondrial damage [9]; elevated levels have been reported in experimental models and in intraocular fluids of patients with PDR [10]. IL4, a key cytokine of the Thelper type 2 (Th2) response, antagonizes several proinflammatory pathways [11] and is considered a potential immunomodulatory therapeutic target beyond ophthalmology, although its role in DR remains contextdependent. IL6 is a pleiotropic cytokine with both pro and antiinflammatory actions, serving as a marker of neuroinflammation and endothelial dysfunction [12]. In DR, higher aqueous humor (AH) levels of IL6 are associated with the presence/severity of DME and overall disease activity and, in some reports, with poorer functional outcomes of therapy [12-14]. Finally, IL8 (CXC motif chemokine ligand 8, CXCL8) is a potent chemoattractant with proangiogenic activity; its concentrations are elevated in serum, AH, and vitreous in PDR/DME and correlate with retinal edema and vascular remodeling [15, 16]. Taken together, these data support the concept of a cytokine imprint of the ocular microenvironment that reflects the activity of proinflammatory and proangiogenic processes across DR phenotypes.

Local intraocular biofluids are indispensable matrices for quantifying these markers. Among them, the AH is the most readily accessible medium proximal to the posterior segment; its composition reflects tissue and celllevel processes within the retina and choroid [17]. Multiomics investigations of AH in DR/DME have demonstrated shifts in metabolic profiles and immunoinflammatory pathways, including elevations in multiple cytokines and extracellularmatrix remodeling factors [18]. Proteomic studies have identified dozens of candidate proteins that correlate with DR severity and its complications [19, 20]. The concept of an ocular "liquid biopsy" has been convincingly validated in retinoblastoma, where AH outperformed blood with respect to tumorderived DNA and protein biomarkers [21].

In view of the above, we selected interleukins as local biochemical indicators of DR progression. Published evidence indicates that IL6 and IL8 in AH most consistently associate with the presence of DR/DME; however, robust stagespecific thresholds have not yet been established [16, 22]. Against this background, AH may be considered a local molecular mirror of hypoxicinflammatory processes in the posterior segment – particularly the retina – in DR, complementing OCTAderived perfusion metrics in the DCP and other retinal layers to enable integrated risk stratification [4, 5].

AIM

Objective – to determine the concentrations of interleukins (IL1β, IL4, IL6, IL8) in the aqueous humor (AH) and their association with diabetic retinopathy (DR) severity.

MATERIALS AND METHODS

All procedures conformed to the Council of Europe Convention on Human Rights and Biomedicine and to the principles of the Declaration of Helsinki (1964, with subsequent amendments including the 2000 revision) and complied with Ukrainian legislation. The study protocol was approved by the Bioethics and Academic Integrity Committee of Bogomolets National Medical University (Protocol No. 196, 23 June 2025). The study was a singlecenter, crosssectional analysis with prospective biospecimen collection.

We enrolled 110 patients with T2DM and DR; analyses were based on the worstaffected eye (110 eyes). According to the ICDR severity scale [3], participants were allocated to five groups: (1) no retinopathy (DR0), n=15; (2) mild NPDR, n=40; (3) moderate NPDR, n=25; (4) severe NPDR, n=12; and (5) PDR, n=18.

Participants were 50-76 years old (median 62.5 [51.3-69] years); men: 92 (83.6%), women: 18 (16.4%), with no betweengroup sex differences (p=0.970). DME was present in 12/110 (10.9%) patients, of whom 10 (83.3%) belonged to groups 4 and 5. A control cohort of 25 age and sexcomparable individuals without diabetes or DR, undergoing surgery for agerelated cataract, was also included.

All participants underwent standardized ophthalmic evaluation, including distance visual acuity testing with a premium test chart projector (C.S.O. srl., USA) using Optiek XL trial lenses (USA) and a Takagi VT5 phoropter (Takagi Seiko Co., Ltd., Japan); autorefraction (TOPCON KR7000P; TOPCON Corporation, Japan); noncontact tonometry (Huvitz HNT7000) and keratopachymetry (HNT1P; Huvitz, Korea); slitlamp biomicroscopy (CSO SL9900 with LED 5× video system; Italy) and binocular

Table 1. Aqueous humor interleukin concentrations by study group (Me; Q1–Q3)

Analyte	Study group						p
	Control	1st (DR0)	2nd (NPDR1)	3rd (NPDR2)	4th (NPDR3)	5th (PDR)	
IL-1b, pg/mL	2,12^{abcde} (0,89–2,49)	3,99^{0de} (3,16–5,21)	4,04^{0de} (2,77–5,68)	5,46^{0e} (40,3–6,32)	9,05^{0ab} (7,59–11,04)	21,86^{0abc} (17,2–28,33)	<0,001
IL-4, pg/mL	9,22^{bcde} (6,89–10,52)	5,24^{cde} (4,68–7,47)	4,64^{0ce} (3,75–5,74)	2,67^{0ab} (2,12–3,96)	2,41^{0a} (1,44–3,56)	1,1^{0ab} (0,64–1,43)	<0,001
IL-6, pg/mL	15,7± 7,7^{bcde}	29,1± 11,8^{de}	38,5± 13,7^{0de}	47,2± 14,7^{0de}	129,2± 78,7^{0abce}	314,3± 68,9^{0abcd}	<0,001
IL-8, pg/mL	34,8^{cde} (27,2–41,6)	45,2^{cde} (27,8–54,3)	57,4^{cde} (37,6–74,9)	143,7^{0ab} (113,1–182,9)	350,1^{0ab} (309,7–369,8)	269,4^{0ab} (226,6–301,9)	<0,001

Notes: Betweengroup comparisons used ANOVA (for normally distributed data) or the Kruskal–Wallis test (for nonnormal data); posthoc testing employed Tukey-Kramer or Dunn's tests, respectively:
[0] – statistically significant differences versus control group, p<0,05;
[a] – statistically significant differences versus 1st group, p<0,05;
[b] – statistically significant differences versus 2nd group, p<0,05;
[c] – statistically significant differences versus 3rd group, p<0,05;
[d] – statistically significant differences versus 4th group, p<0,05;
[e] – statistically significant differences versus 5th group, p<0,05.

indirect ophthalmoscopy (Heine OMEGA 600 Traveler Set; HEINE, Germany); gonioscopy using a contact threemirror Optiek XL lens (USA); ophthalmoscopy with Volk Digital Wide Field lenses (Germany) and a Goldmann threemirror lens; and optical coherence tomography with fundus camera and angiography (HOCT1F, Huvitz Co. Ltd., Korea).

At the start of phacoemulsification, 0.1 mL of AH was obtained via anteriorchamber paracentesis. Concentrations of IL1β, IL4, IL6, and IL8 (pg/mL) were measured by solidphase ELISA using Invitrogen (Thermo Fisher Scientific, USA) kits.

Statistical analyses were performed in EZR v.1.54 (graphical interface to R v.4.0.3; R Foundation for Statistical Computing, Austria) [23]. Because data deviated from normality, results are reported as medians (Me) with interquartile ranges (Q1-Q3). Group comparisons used the Kruskal-Wallis test with Dunn's post hoc procedure; α=0.05 [24]. Diagnostic cutoffs for interleukin concentrations were derived using multiclass classification (onevsall approach) with ROCbased performance assessment [25, 26].

ETHICS STATEMENT

This study involved human participants and was approved by the local bioethics committee. Written informed consent was obtained from all participants. The research was conducted in accordance with the Declaration of Helsinki. No animal experiments were performed.

RESULTS

Analyses were performed across five clinical groups of patients with T2DM who either had no DR or exhibited DR at various stages (mild, moderate, severe NPDR, or PDR). Sex distribution was balanced across groups (women: 18 (16.4%); men: 92 (83.6%); p=0.9703), minimizing sex as a potential confounder. Age was comparable between groups (global p=0.108), whereas diabetes duration increased in a stepwise fashion with DR stage: DR0 – 5.0 (3.3-7.5) years; NPDR1 – 10.0 (5.0-14.5); NPDR2 – 14.0 (10.0-18.5); NPDR3 – 16.5 (10.0-21.0); PDR – 15.5 (15.0-25.0); $p<0.001$.

At enrollment, indices of carbohydrate metabolism (fasting plasma glucose, HbA1c) did not differ significantly across groups (p=0.176 and p=0.101, respectively), whereas Cpeptide showed a downward trend in more severe phenotypes without reaching statistical significance (p=0.108). Thus, the clinical groups were comparable for age, sex, and glycemic control and differed primarily by diabetes duration – parameters that define the baseline context for subsequent analyses of AH interleukin levels.

Table 1 summarizes the concentrations of IL1β, IL4, IL6, and IL8 in AH by group. Global betweengroup differences were statistically significant for all markers (all p<0.001) and were corroborated by appropriate

Table 2. Analytical performance of stage prediction according to aqueous humor interleukin concentrations at different stages of diabetic retinopathy

Metric	Study group					
	Control	DR0	NPDR1	NPDR2	NPDR3	PDR
IL-1β, pg/mL						
Cutoff	<2.98	2.98-6.21	6.22-7.87	7.88-8.35	8.36-13.68	>13.68
Sensitivity, %	100	73.3	17.5	4.0	75.0	100
Specificity, %	84.5	77.5	91.6	100	100	99.1
Overall accuracy, %	53% (CI 44% – 62%)					
IL-4, pg/mL						
Cutoff	>5.98	5.98-4.20	4.19-3.10	3.11-1.84	1.83-0.65	<0.65
Sensitivity, %	96.0	46.7	20.0	40.0	41.7	27.8
Specificity, %	83.6	90.0	92.6	93.6	86.2	96.6
Overall accuracy, %	44% (CI 35% – 53%)					
IL-6, pg/mL						
Cutoff	<23.8	23.8-34.3	34.4-67.0	67.1-86.2	86.3-288.5	>288.5
Sensitivity, %	92.0	46.7	62.5	0	58.3	72.2
Specificity, %	90.0	90.0	69.5	100	95.9	100
Overall accuracy, %	56% (CI 47% – 64%)					
IL-8 pg/mL						
Cutoff	<54.7	54.7-70.3	70.4-113.8	113.9-256.5	256.6-351.2	>351.2
Sensitivity, %	100	26.7	32.5	76.0	50.0	66.7
Specificity, %	70.2	94.7	94.4	96.2	100	96.6
Overall accuracy, %	58% (CI 49% – 67%)					

post hoc testing (Tukey-Kramer or Dunn's, according to distributional assumptions).

For IL1β, a monotonic increase was observed from control to PDR: control, median 2.12 pg/mL (0.89-2.49); PDR, 21.86 pg/mL (17.2-28.33), with sequential stepups between intermediate classes.

IL4 decreased with increasing DR severity: control, 9.22 pg/mL (6.89-10.52) to PDR, 1.10 pg/mL (0.64-1.43), a pattern compatible with a shift away from a Th2type immune profile as DR progresses.

IL6 exhibited the largest dynamic range (mean±SD), rising from 15.7±7.7 pg/mL in controls to 314.3±68.9 pg/mL in PDR, with a pronounced surge at NPDR3 (129.2±78.7 pg/mL).

For IL8, a characteristic "peaked" pattern emerged: values increased from control 34.8 pg/mL (27.2-41.6) to NPDR3 350.1 pg/mL (309.7-369.8), followed by a partial decline in PDR to 269.4 pg/mL (226.6-301.9), consistent with a chemoattractant/proangiogenic axis predominating at nonproliferative stages and transitioning into a remodeling phase thereafter [27].

To analyze AH interleukin levels across DR stages and to evaluate their association with stagewise progression, we selected optimal thresholds using a OnevsAll multiclass classification approach [25].

The visualization in Figure 1 revealed orderly yet distinct gradients: IL1β increased monotonically from control to PDR; IL4 declined with advancing severity; IL6 showed the largest dynamic range with a marked surge at NPDR3; and IL8 exhibited a "peaked" profile with a maximum at NPDR3 followed by a relative decrease in PDR.

These visual trends informed a sixclass classification scheme (control, DR0, NPDR1, NPDR2, NPDR3, PDR), for which Table 2 provides cutoff intervals and classspecific metrics (sensitivity/specificity) together with overall model accuracy.

IL1β cutoffs: <2.98 (control), 2.99-6.21 (DR0), 6.22-7.87 (NPDR1), 7.88-8.35 (NPDR2), 8.36-13.68 (NPDR3), >13.68 pg/mL (PDR). Very high specificity was observed at the extremes (notably PDR), but overlap between adjacent NPDR classes yielded an overall accuracy of ≈ 53% (95% CI: 44-62%).

IL4 cutoffs shifted downward from higher values in controls to lower values in PDR (from >5.98 to <0.65 pg/mL), with overall accuracy ≈ 44% (35-53%).

IL6 cutoffs: <23.8 (control), 23.8-34.3 (DR0), 34.4-67.0 (NPDR1), 67.1-86.2 (NPDR2), 86.3-288.5 (NPDR3), >288.5 pg/mL (PDR), with overall accuracy ≈ 56% (47-64%).

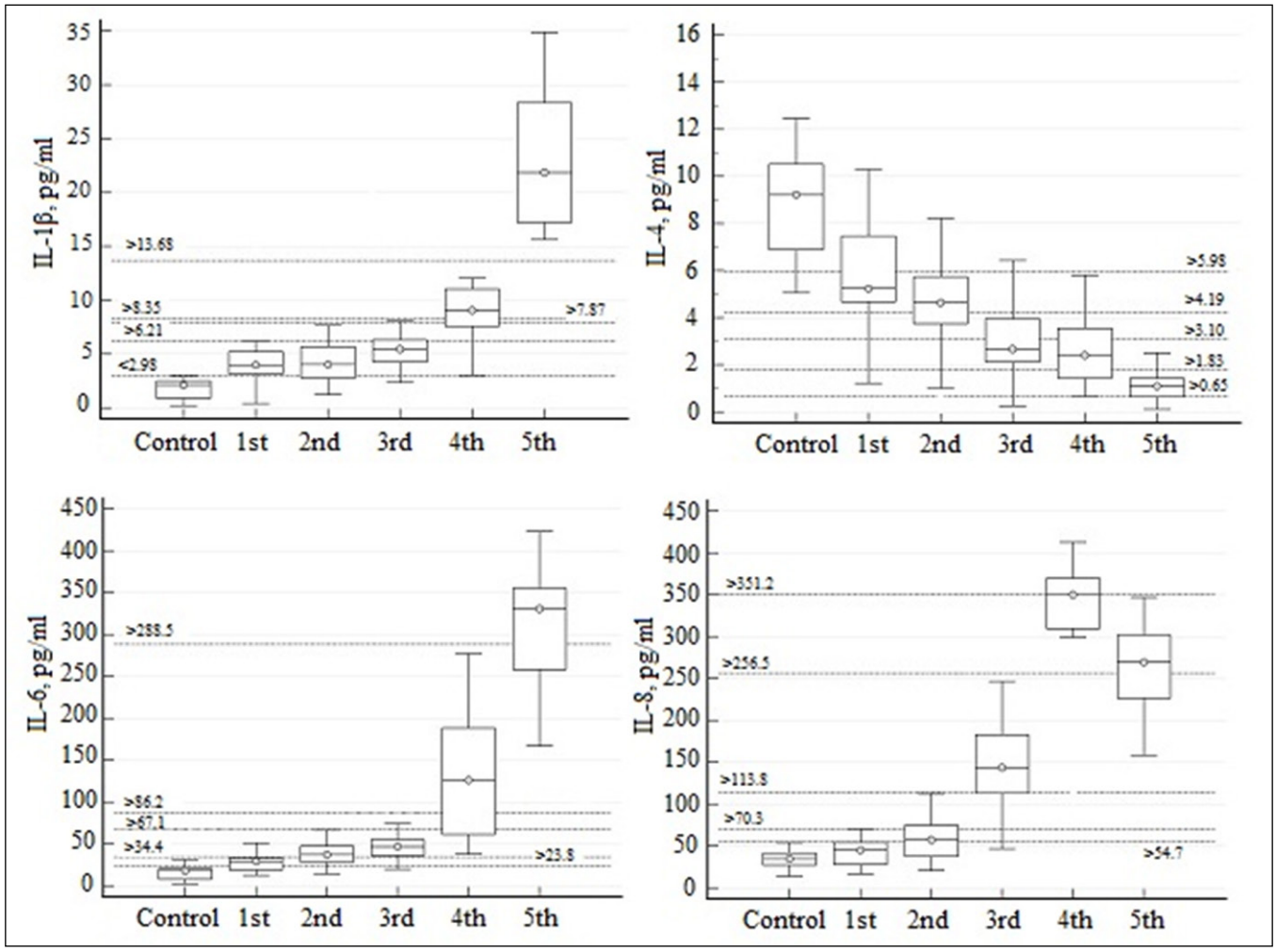

Figure 1. Aqueous humor interleukin concentrations by group; yaxis: interleukin concentration (pg/mL). Cutoff values (pg/mL) used for DR stage prediction are indicated on the plots.

IL8 cutoffs respectively: <54.7, 54.3-70.3, 70.4-113.8, 113.9-256.5, 256.6-351.2, >351.2 pg/mL, with overall accuracy ≈ 58% (49–67%).

Thus, in the sixclass formulation, all four interleukins provided better metrics at the extremes (control, PDR) due to high specificity, with expected distributional overlap among adjacent NPDR categories that limited classbyclass discrimination (Figure 1, Table 2).

To account for the continuous biology of DR progression and the discrete nature of the ICDR clinical scale - and to improve analytical performance - we applied clinically relevant binarization of phenotypes into two integrated groups: mild/moderate DR (NPDR1 + NPDR2) and severe DR (NPDR3 + PDR) (Figure 2).

On Figure 2 the binary thresholds are summarized, while Table 3 reports their analytical characteristics (sensitivity, specificity, accuracy). Among the markers, IL6 performed best: 34.4–86.2 pg/mL corresponded to mild/moderate DR, whereas > 86.2 pg/mL indicated severe DR; overall accuracy was 72.6% (95% CI 64.3–79.9) with an acceptable balance of sensitivity and specificity.

For IL8, the corresponding thresholds 70.4-216.9 and > 216.9 pg/mL yielded 62.2% (95% CI 53.2-70.7) accuracy, supporting its role as an auxiliary marker in the binary model. By contrast, IL1β (6.22-8.24 and > 8.24 pg/mL) and IL4 (1.83-4.18 and < 1.83 pg/mL) each achieved ≈ 55.6% accuracy, indicating limited practical utility for distinguishing mild/moderate from severe DR.

Taken together, Figure 2 and Table 3 illustrate the translation of continuous interleukin changes into a parsimonious prognostic scale, with IL6 emerging as the primary single local biomarker for rapid risk stratification.

To align the biochemical profile with the clinicalmetabolic context, we computed Spearman rank correlations between AH interleukin levels and age, diabetes duration, fasting glucose, HbA1c, and Cpeptide (Figure 3).

The expected pattern of weaktomoderate correlations emerged: positive associations for inflammatory/angiogenic markers – particularly IL6 and IL8 – with diabetes

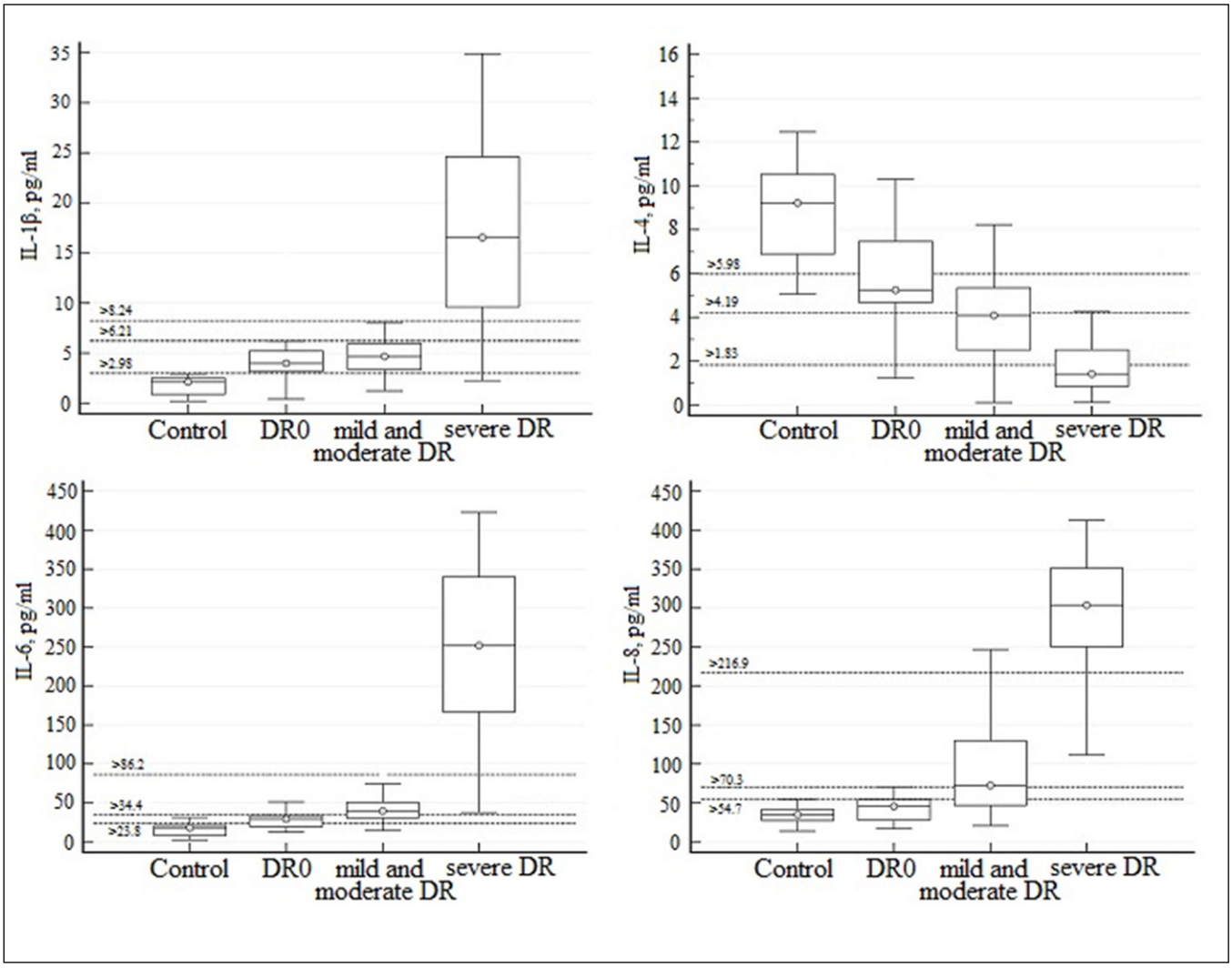

Figure 2. Threshold interleukin levels in aqueous humor (integrated groups); yaxis: interleukin concentration (pg/mL).
Cutoff values (pg/mL) are shown for predicting mild and moderate DR (NPDR2 and NPDR3 groups) and severe DR (NPDR3 and PDR groups).

duration and selected indices of carbohydrate metabolism, and negative associations for IL4 and Cpeptide (as an indicator of βcell reserve). Several pairs reached $p<0.05$; however, the absolute magnitudes remained within the weak/moderate range, consistent with the local (intraocular) nature of the markers compared with systemic metrics.

This correlation profile strengthens the biologic plausibility of the proposed thresholds: as diabetes duration increases and/or glycemic control deteriorates, neuroinflammatory/angiogenic indicators shift toward higher ranges, whereas the regulatory IL4 decreases; at the same time, a substantial portion of variance is extrasystemic, i.e., driven by local ocular processes, further justifying AH as a target matrix for biomarker analysis.

DISCUSSION

Our findings confirm that the AH exhibits a clear proinflammatory/proangiogenic gradient across analytes with advancing DR stage. In a clinically relevant binary framework (mild/moderate vs severe DR), IL6 showed the highest discriminative performance (overall accuracy 72.6%), whereas IL1β and IL4 were weak classifiers in our cohort, and IL8 functioned as an auxiliary marker with better specificity at the extremes. This hierarchy accords with contemporary concepts on the central role of IL6 in retinal microvascular dysfunction – including transsignaling via sIL6R (soluble interleukin 6 receptor)/gp130 (glycoprotein 130) – which integrates neuroinflammation, endothelial hyperpermeability, and hypoxiadriven cascades [28-31].

The biological rationale for this hierarchy is well explained by current insights into NLRP3mediated inflammation, IL6 transsignaling, and neutrophil chemoattraction [32]. Hyperglycemia activates the NLRP3 inflammasome, triggering IL1β and IL18 release, pyroptotic death within the retinal neurovascular unit, and disruption of the blood-retinal barrier (BRB) [32]. These

Table 3. Analytical performance of predictions based on aqueous humor interleukin concentrations (integrated groups)

Metric	DR stage			
	Control	DR0	Mild & Moderate DR	Severe DR
		IL-1β, pg/mL		
Cutoff	<2.98	2.98-6.21	6.22-8.24	>8.24
Sensitivity, %	88.0	73.3	23.1	90.0
Specificity, %	84.5	90.0	98.6	96.2
Overall accuracy, %	55.6% (CI 46.8% – 64.1%)			
		IL-4, pg/mL		
Cutoff	>5.98	4.19-5.98	1.83-4.18	<1.83
Sensitivity, %	96.0	46.7	36.9	66.0
Specificity, %	83.6	91.7	88.6	89.5
Overall accuracy, %	55.6% (CI 46.8% – 64.1%)			
		IL-6, pg/mL		
Cutoff	<23.8	23.8-34.3	34.4-86.2	>86.2
Sensitivity, %	80.0	46.7	70.8	83.3
Specificity, %	90.0	92.5	84.3	100
Overall accuracy, %	72.6% (CI 64.3% – 79.9%)			
		IL-8, pg/mL		
Cutoff	<54.7	54.8-70.3	70.4-216.9	>216.9
Sensitivity, %	95.7	26.7	50.8	83.3
Specificity, %	70.2	96.4	91.9	98.1
Overall accuracy, %	62.2% (CI 53.2% – 70.7%)			

pathways – corroborated by experimental data, clinical specimens, and systematic reviews [32-34] – provide the substrate for the pleiotropic actions of IL6 and the proangiogenic/chemokine effects of IL8.

In the literature, IL1β is consistently linked to BRB dysfunction (NFκB activation, oxidative stress, mitochondrial injury) and pyroptosis [35], as well as to pericyte loss [36], a classic early hallmark of diabetic microangiopathy [37]. Although elevated IL1β has been repeatedly documented in retinal tissue and intraocular fluids in experimental diabetes and in patients with PDR, our classification analyses – unsurprisingly – revealed overlap of IL1β concentrations between adjacent NPDR stages, leading to low overall accuracy despite high specificity at the extremes (DR0, PDR). Thus, IL1β emerges as a sensitive indicator of retinal inflammatory activity but not a reliable stratifier of DR severity.

A similar rationale applies to IL4. As a Th2 associated immunomodulator, IL4 has been shown to protect pericytes, reduce endothelial permeability, and modulate the microglial response via signal transducer and activator of transcription6 (STAT6)-dependent mechanisms [38]. However, in realworld clinical settings – amid variability in glycemia, diabetes duration, and other systemic parameters – the antiinflammatory axis of IL4 manifests as a general downward trend without a clear demarcation between adjacent stages. Consequently, within a binary severity framework, IL4 offers limited value as a "rulein/ruleout" marker despite its evident biological relevance.

The literature contains substantial evidence linking intraocular IL6 with DR/DME phenotypes, reinforcing the external plausibility of our conclusion that IL6 is the priority biomarker for binary stratification. Recent reviews consistently demonstrate associations between elevated IL6 in aqueous/vitreous humor and retinal edema, microvascular disorganization, and concordant imaging changes, underscoring the conceptual nodal role of IL6 along the DR-DME pathobiologic axis [39, 40]. In addition, multiomics studies of AH delineate protein networks in which IL6 occupies a central position among inflammatory and extracellularmatrix remodeling mediators, aligning with our empirical identification of IL6 as the most informative single local indicator of DR severity for a clinically relevant twoclass model [31,42]. Collectively, these data support the use of IL6 threshold intervals as a practical instrument for risk stratification in routine clinical scenarios [40].

The stagedependent behavior of IL8 in our cohort – marked elevation through NPDR3 with a relative decrease

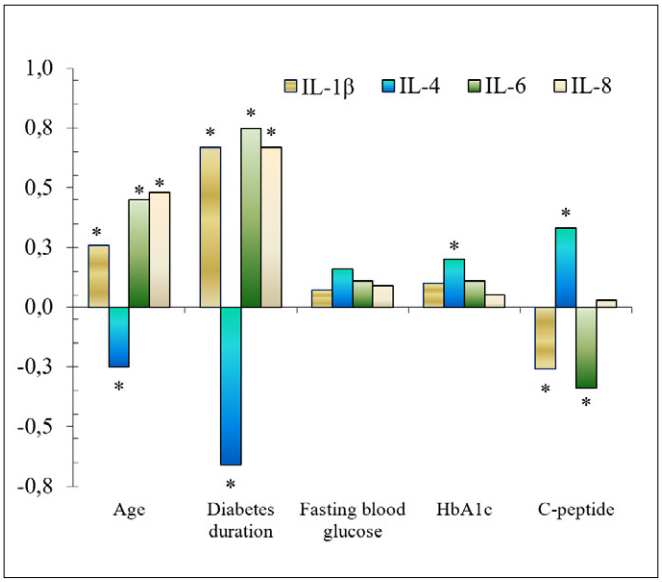

Figure 3. Spearman rank correlation coefficients for AH interleukins versus age, diabetes duration, fasting blood glucose, HbA1c, and Cpeptide; yaxis: correlation coefficient. * indicates p<0.05.

in PDR – explains why it underperforms IL6 in binary metrics despite strong specificity at the extremes. Broader transitional ranges between adjacent classes produce overlap and reduce overall accuracy. This pattern accords with aggregated clinicallaboratory observations in which IL8 consistently associates with inflammatory activity, edema severity, and microvascular remodeling, more reliably flagging phases of maximal activity than faithfully mirroring gradations of process severity across DR [22, 43].

The conceptual framework for interpreting our molecular thresholds is their concordance with quantitative imaging of retinal microperfusion. OCTA data indicate that the earliest alterations are localized to the DCP, characterized by perivenular capillary rarefaction and an expansion of nonperfusion, which correlate with the occurrence of complications and with DR progression [44]. Moreover, in a subset of patients, applied OCTA metrics – such as the algorithmic diabetic macular ischemia (DMI) index, branching/fragmentation indices, and foveal avascular zone (FAZ)derived parameters – carry prognostic value [45-49].

Against this backdrop, IL6 threshold intervals can serve as a molecular complement to the structural-perfusion signal: OCTA tracks the consequences of microvascular dysfunction, whereas the AH composition captures the active biochemical processes sustaining it. Integrating these domains thus provides the basis for individualized risk assessment of DR progression within a unified clinical strategy [50].

The correlation analysis merits separate consideration as a tool for aligning local AH interleukin dynamics with the systemic background of DR (age, diabetes duration, fasting glucose, HbA1c, Cpeptide). The observed weaktomoderate correlations are methodologically expected: in biomedical datasets, correlation coefficients are seldom large and should be interpreted not solely by ρ, but primarily through effect size and clinical significance [51, 52]. More broadly, published correlational effect sizes in clinical sciences tend to cluster within the small/moderate range [53], underscoring the value of combining molecular and imaging indicators to enhance prognostic sensitivity.

Substantively, the positive correlations of IL6/IL8 with diabetes duration and with selected glycemic indices mirror established epidemiological patterns: disease duration and metabolic milieu (including HbA1c variability) are associated with DR risk/progression, although their direct influence on local intraocular mediators remains incompletely defined [54-56]. Conversely, the inverse correlations with Cpeptide – characteristic of regulatory parameters in our sample – are consistent with reports on the prognostic relevance of postprandial Cpeptide levels and Cpeptide-to-glucose ratios for DR risk stratification in type 2 diabetes [57, 58]. Importantly, these systemic factors explain only a limited proportion of the variability in intraocular interleukin levels; the major contribution arises from local ocular processes, as supported by contemporary profiling studies [42].

A key methodological anchor of our approach is its continuity with previously validated local biomarkers in aqueous humor within the same disease model, particularly HIF1α as an indicator of hypoxic load: an analogous thresholdbased and binary framework provided practical utility for severity stratification in our prior work [17]. This concordance strengthens the credibility of interpreting local threshold readouts and furnishes a rationale for their subsequent integration into a composite diagnostic panel.

Practical implications for clinical management can be summarized as follows. First, IL6 should be considered a primary local risk indicator in patients with DR: values above the upper bound of the mild/moderate interval in our dataset (>86.2 pg/mL) support classification into the severe DR group and justify shorter followup intervals with targeted OCT/OCTA surveillance, whereas values within the mild/moderate interval may support standard visit frequency with emphasis on optimizing systemic metabolic risk factors for DR.

Second, IL8 may be used as an adjunct to corroborate polar phenotypes; however, midspectrum interpretation warrants caution because of broader interclass overlap.

Third, IL1β and IL4 do not provide decisive standalone information in the binary framework, limiting their use as solitary cutoffs; their application appears more

promising within combined panels subject to external validation [47,50].

Strengths of the study include a singlecohort design with consistent AH sampling, centralized and standardized preanalytics within one center, and a harmonized statistical framework (threshold intervals; multiclass and binary models with estimates of sensitivity, specificity, and overall accuracy), all of which ensured internal coherence of conclusions and reproducibility of the interpretive logic. Corroboration by independent sources (reviews/analyses on interleukins, AH proteomics, and OCTA metrics) enhances the external validity of the key conclusion prioritizing IL6 in binary stratification [39, 41, 45].

Limitations include the singlecenter, crosssectional design without evaluation of downstream clinical outcomes, which constrains causal inference and limits generalizability of numeric thresholds. Class imbalance across stages (uneven group representation) may influence the stability of certain metrics – particularly accuracy – in external datasets, despite the invariance of ROC curves to class prevalence; hence the need for external testing in representative cohorts [59, 60].

Results may also be affected by preanalytical factors (storage duration/conditions, freeze–thaw cycles) and crossplatform differences among immunoassay methods (ELISA, multiplex systems). Although unified procedures were followed, contemporary guidance recommends protocol standardization and interlaboratory comparison prior to routine implementation of quantitative cutoffs [61-63]. Finally, the absence of longterm followup precludes assessment of the prognostic value of the proposed thresholds for clinically meaningful endpoints (progression to NPDR3/PDR, development of DME), defining priorities for future multicenter prospective studies with independent external validation [64].

CONCLUSIONS

1. We observed significant betweengroup differences in AH interleukin levels (all $p<0.001$): IL1β and IL6 increased with advancing stage; IL8 peaked at NPDR3 with a relative decline in PDR; IL4 progressively decreased as DR progressed.
2. In the multiclass model, singleanalyte performance yielded only moderate overall accuracy (IL6 ≈56%, IL8 ≈58%, IL1β ≈53%, IL4 ≈44%), with the best discrimination at the extremes – findings that supported a shift to binary stratification.
3. In the binary model (mild/moderate vs severe DR), IL6 demonstrated the highest informativeness (accuracy 72.6%, 95% CI 64.3-79.9), IL8 provided auxiliary value (62.2%), whereas IL1β and IL4 were of limited practical utility for classification.
4. Operational cutoffs for clinical management were established: for IL6 – 34.4-86.2 pg/mL (mild/moderate DR) and >86.2 pg/mL (severe DR); for IL8 – >216.9 pg/mL as a supportive indicator of severe DR.
5. Correlation analysis confirmed weaktomoderate positive associations of IL6/IL8 with diabetes duration and selected glycemic indices, and inverse correlations with Cpeptide, characteristic of regulatory parameters. This profile reflects the local nature of AH mediators and supports the biological plausibility of the proposed thresholds.
6. IL6 can serve as a primary local risk marker to identify patients with a high likelihood of severe DR who warrant shortened followup intervals and intensified structural-functional surveillance using OCTA. Next steps include external validation of numeric cutoffs, preanalytical standardization, and prospective studies evaluating clinically meaningful endpoints.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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