Vitreous cytokine expression profiles in patients with retinal detachment

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VITREOUS CYTOKINE EXPRESSION PROFILES IN PATIENTS WITH RETINAL DETACHMENT

Short title: Expression profiles of cytokines in patients with retinal detachment

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ABSTRACT

Purpose: To compare the expression profiles of various cytokines and chemokines in vitreous samples from patients with retinal detachment (RD) to those from controls and to analyze their association with different clinical features.

Methods: In this prospective study, undiluted vitreous fluid was obtained from 41 patients with primary RD and 33 controls with macular hole or vitreomacular traction. A multiplex bead immunoassay was performed to determine the expression of 27 inflammatory mediators.

Results: Eleven mediators were significantly upregulated in the vitreous from RD patients compared with controls, including the following: cytokines IL-1ra, IL-6, IL-7, IL-8, IFN-γ; chemokines CCL2, CCL3, CCL4, CXCL10 and CCL11 and growth factor G-CSF. Correlation analyses showed that levels of IL-1ra, CXCL10, CCL11 and G-CSF were positively correlated to the extent of detachment while those of IL-1ra and CXCL10 were associated with the duration of detachment. There was also a positive association between the concentrations of CXCL10 and CCL11 and preoperative flare values. Additional analysis revealed that flare values and both CXCL10 and CCL11 levels were significantly higher in eyes with grade B or C proliferative vitreoretinopathy (PVR).

Conclusion: Our results confirm that RD induces a marked inflammatory response with a complex cytokine network. We identified proteins specifically linked to several clinical features that might contribute to photoreceptor degeneration and PVR redetachment. They may represent potential therapeutic targets for improving the anatomical and functional outcomes of RD surgery.

Keywords: cytokine, retinal detachment, aqueous flare, proliferative vitreoretinopathy

INTRODUCTION

Rhegmatogenous retinal detachment (RD) is a sight-threatening condition with an annual incidence of approximately 10 per 100,000 people [1]. Advances in surgical techniques over recent decades have greatly improved anatomical results with a primary success rate currently up to 80% [2]. However, despite a successful retinal reattachment, visual recovery may still be disappointing after RD involving the macula and this loss of vision is primarily due to photoreceptor cell death [3,4]. The physical separation of the neurosensory retina from the underlying retinal pigment epithelium (RPE) and choroidal vasculature impairs the oxygen and nutrient supply to the photoreceptor cells, which eventually die mainly through apoptosis [5–8]. Several pathogenic mechanisms have been identified but accumulating evidence suggests that inflammation plays a key role in the pathogenesis of RD-induced photoreceptor cell death. Human studies have thus reported elevated levels of cytokines and chemokines in the aqueous humor, vitreous and subretinal fluid from eyes with RD [9–15]. Furthermore, experimental models have demonstrated that infiltrating immune cells and associated inflammatory mediators directly induce the death of photoreceptors following RD [16–18].

Inflammation has also been shown to contribute to the development of proliferative vitreoretinopathy (PVR), which remains the most common cause of failure after RD repair [19]. The process of PVR has many similarities to a wound-healing response involving migration and proliferation of resident ocular cells and invading immune cells, and the production of local factors [19]. This ultimately leads to the formation of epiretinal and subretinal membranes that may cause retinal redetachment upon contraction. Several studies have thus found macrophages present in the vitreous and inside the retinal tissue in human PVR samples [19–22]. Moreover, it has been demonstrated that the presence of these cells is associated with a high risk of developing PVR [19,23]. A wide variety of

cytokines have also been shown to be upregulated in ocular fluids from eyes with established PVR and have therefore been proposed as predictive biomarkers for later PVR redetachment.

However, these mediators are not specific for PVR and do not differ from those observed in any RD. Besides, these measurements require samples and laboratory analysis and are therefore difficult to apply in clinical practice. In contrast, quantification of aqueous flare in the anterior chamber with a laser flare-cell meter provides a fast and safe means to measure aqueous protein concentration and to assess the breakdown of the blood-retinal barrier in RD [24,25]. We and others have recently demonstrated that it may serve as a strong preoperative predictor for PVR development and that elevated flare values correspond to an altered profibrotic intraocular cytokine milieu [26–28].

Although many studies have investigated the intraocular cytokine/chemokine profiles in RD patients, little is known about the clinical parameters that might influence the expression of these molecules. Yet, this question seems of utmost importance to understand the pathophysiological mechanisms in RD and notably those underlying the photoreceptor degeneration and occurrence of PVR.

The aim of this study was to assess the levels of various cytokines and chemokines in vitreous samples from patients with RD compared to controls with macular hole and to correlate these levels with different clinical variables including aqueous flare values.

METHODS

Study design

We conducted a prospective nonrandomized clinical trial at Nancy University Hospital from November 2017 to August 2018. This study was approved by the regional Institutional Ethics Committee (Comité de Protection des Personnes CPP17-059/2017-A02195-48) and adhered to the tenets of the Declaration of Helsinki. This study was also registered in the database of the National Institutes of Health at clinicaltrials.gov (identification number NCT03318588).

Participants

Consecutive eligible patients were offered participation in the trial.

Inclusion criteria for the RD group were as follows: 1) patients over 18 years of age, 2) presenting with primary RD, 3) requiring vitrectomy. Inclusion criteria for the control group were as follows: 1) patients over 18 years of age, 2) presenting with vitreomacular traction (VMT) or macular hole (MH), 3) requiring vitrectomy. Non-inclusion criteria were a history of vitreoretinal surgery, traumatic RD or any concomitant retinal pathology such as proliferative diabetic retinopathy, diabetic macular edema, age-related macular degeneration, retinal dystrophies or retinal vein occlusion. Patients were also not eligible for the study if they had preoperative vitreous hemorrhage or systemic comorbidities that might potentially influence intraocular cytokine levels. All patients had complete information about the study and the risks and benefits of the surgical procedure and gave their written consent for participation before surgery.

All patients underwent a detailed ophthalmologic examination before and after surgery, including best-corrected visual acuity (BCVA) measured with projected-light Snellen charts, axial length measurement using IOLMaster (Carl Zeiss Meditec, Dublin, CA),

biomicroscopy with anterior segment evaluation, fundus and careful peripheral retina examination.

In the RD group, an Amsler-Dubois scheme was systematically established for each patient, specifying the extent of the RD, number, type and location of retinal breaks, existence of vitreous hemorrhage and preoperative proliferative vitreoretinopathy (PVR) grading according to Machemer et al. [29]. Aqueous flare was measured with the Kowa FM-500 Laser Flare-Cell Meter (Version 1.0; Kowa Company Ltd, Tokyo, Japan) 30 minutes after the instillation of 0.5% tropicamide and 5% phenylephrine hydrochloride. Ten measurements were taken and averaged from each affected eye. Measurements with artifacts were excluded. Flare values were expressed as photon counts per millisecond (pc/ms).

Patients were systematically examined within the first week after surgery and then at 1 and at least 6 months postoperatively. Additional examinations were provided when needed.

Demographic data, axial length, lens status, characteristics of RD, BCVA and preoperative aqueous flare values were documented. We also collected the primary and final anatomical success rate, causes of failure and postoperative BCVA in the RD group.

Surgical technique

All patients underwent a three-port 23- or 25-gauge pars plana vitrectomy. At the beginning of the vitrectomy, air perfusion was set to open and undiluted vitreous fluid samples (1 mL) were collected from each eye with 3 mL syringe. Samples were sent to the Biological Resource Center (Centre de Ressources biologiques, Nancy, France) within 30 min,

cooled on ice and transferred into microfuge tubes. Each sample was centrifuged at 10,000 g for 5 min, and the supernatant was then collected and frozen at -80 °C before analysis.

In the RD group, an extensive vitrectomy was then performed followed by fluid-air exchange, retinopexy with either endophotocoagulation or cryotherapy and gas (25% SF6 or 20% C2F6/air mixture) or silicone oil tamponade depending on the surgeon's preferences, based on clinical criteria including location, size and number of retinal breaks, extent of RD, existence of preoperative PVR or vitreous hemorrhage. In the control group, the ILM was systematically removed, and a 25% SF6/air mixture was used for tamponade. After surgery, patients were instructed to avoid the supine position during the night for a minimum of 1 week.

Multiplex bead immunoassay

Inflammatory cytokine concentrations were measured using Bio-Plex Pro™ Human Cytokine 27-plex Assay (Bio-Rad Laboratories, Marnes-la-Coquette, France). Briefly, the vitreous samples were thawed and diluted twofold through the use of the dilution solution provided in the Bio-Plex beads array kit. Cytokine levels were measured in duplicate with 50 µL of diluted supernatant in accordance with the manufacturer's instructions. The following 27 cytokines and chemokines were targeted: IL-1ß, IL-1 receptor antagonist (IL-1ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8 [chemokine C-X-C motif ligand (CXCL)8], IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin [chemokine C-C motif ligand (CCL)11], basic fibroblast growth factor (b-FGF), granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GMCSF), interferon (IFN)-γ, interferon-inducible 10-kDa protein [IP-10 (CXCL10)], MCP-1 (CCL2), macrophage inflammatory protein-1 [MIP-1α (CCL3)], MIP-1ß (CCL4), platelet-derived growth factor (PDGF), regulated upon activation, normal T cell expressed and secreted [RANTES (CCL5)], tumor necrosis factor (TNF)- α, and VEGF.

Main outcome measures

The primary endpoint of this study was the cytokine expression profile in the vitreous from RD patients. We also examined the interactions between the upregulated proteins in RD patients and their association with various clinical features.

Statistical analysis

Snellen visual acuity was converted to logarithm of the minimum angle of resolution (logMAR) units. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as numbers and percentages.

Adjusted means of protein concentrations for age, lens status, sex and axial length were computed for each group through propensity score analysis and their difference against 0 was tested using proc mixed procedure. Pearson's correlation coefficients were calculated to analyze the interactions between the upregulated proteins in RD patients and to explore the association between the protein levels and the clinical features based on **continuous or discrete** variables. **Association between protein levels and categorical variables (including ordinal ones) was assessed by using the Mann-Whitney-Wilcoxon test.** All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC 25513). The threshold for statistical significance was set at $p<.05$.

RESULTS

Forty-one eyes of 41 patients were included in the RD group and 33 eyes of 33 patients in the control group.

Baseline characteristics

Baseline characteristics for RD **and control** patients are given in Table 1.

Twenty-seven (65.9%) men were included in the RD group and 7 (21.2%) men in the control group ($p<0.001$). The mean age of RD patients was significantly lower than that of controls: 62.4 ± 12.4 years compared with 72.2 ± 9.1 years ($p<0.001$). Seventeen (41.5%) and 22 (66.7%) eyes of the RD and control groups were phakic at the time of diagnosis ($p=0.031$). There was no difference between the 2 groups in terms of axial length ($p=0.477$).

The mean duration of symptoms before surgery was 7.7 ± 7.3 days with a median of 4.5 days [1-30] in RD patients. The mean extent of RD was 2.1 ± 0.8 quadrants with macular involvement in 65.9% of cases and grade B or C PVR in 36.6% of cases.

Postoperative data

A complete retinal reattachment was achieved in 36 (87.8%) eyes with a single procedure and in 39 (95.1%) eyes with two or more procedures. The causes of primary failure were postoperative PVR in three eyes and new retinal breaks in two eyes.

The mean BCVA significantly improved from 1.2 ± 0.9 logMAR at baseline to 0.3 ± 0.2 logMAR at the end of the follow-up period ($p<0.001$).

Outcomes

Intravitreal cytokine profiles

Of the 27 inflammatory mediators analyzed, 14 were successfully detected and compared between the two groups (Table 2). Out of these and after adjusting for age, sex, lens status and axial length, 11 were significantly increased in the vitreous from RD patients including the following: cytokines IL-1ra, IL-6, IL-7, IL-8, IFN-γ; chemokines CCL2, CCL3, CCL4, CXCL10 and CCL11 and growth factor G-CSF (Table 2). In contrast, the levels of IL-10, IL-13 and VEGF were not statistically different between the two groups (Table 2).

Correlation between upregulated cytokines

Interactions between cytokines in RD patients are given in Table 3.

Most of the cytokines were significantly correlated to each other. Interestingly, CCL2 and IFN-γ were associated with all upregulated cytokines in RD vitreous.

Correlation between cytokine expression and clinical characteristics

The correlation coefficients between the cytokines and the clinical features are summarized in Table 4.

Correlation analyses showed that levels of IL-1ra, CXCL10, CCL11 and G-CSF were positively correlated to the extent of detachment while those of IL-1ra and CXCL10 were associated with the duration of detachment. There was also a positive association between the concentrations of CXCL10 and CCL11 and preoperative flare values. None of the upregulated cytokines was significantly associated with axial length, preoperative BCVA and number of retinal breaks.

Additional analysis revealed that flare values and both CXCL10 and CCL11 levels were significantly higher in eyes with grade B or C PVR ($p<0.001$, $p=0.005$ and $p=0.019$) (Table 5).

DISCUSSION

We carried out a prospective clinical study to assess the expression profiles of intraocular cytokines in RD patients and to examine their association with various clinical features.

Our analysis of vitreous samples shows that RD induces a severe inflammatory response characterized by increased expression of various cytokines, chemokines and growth factors. The expression profile is comparable with that reported in most earlier studies on vitreous, aqueous humor and subretinal fluid from RD patients [9–15], even though it is difficult to make comparisons given the wide variations in terms of the preoperative characteristics of RD and cytokine assay procedures. Interestingly, we found that most of the upregulated cytokines were correlated to each other, suggesting the presence of a complex cytokine network in RD vitreous.

Furthermore, we identified several proteins specifically associated with various clinical features such as duration and extent of detachment and preoperative flare values.

Duration of detachment has been shown to be a major prognostic factor for visual recovery following RD repair [30–32]. Although there is still controversy regarding the exact timing after which visual prognosis may be compromised, we have recently demonstrated that shorter intervals to surgery and better functional outcomes are significantly associated with fewer defects within the photoreceptor layers on SD-OCT [33]. These results are in line with those of experimental studies which found that photoreceptor apoptosis occurred

within hours, peaked at 2 to 3 days, and dropped to a low level 7 days after RD induction [3,4].

There is growing evidence that the innate immune response and notably the mononuclear phagocyte (MP) system, a family of cells including monocytes, inflammatory macrophages, dendritic cells and microglial cells, play a major role in RD-associated photoreceptor death [4,16–18]. Animal studies have indeed shown that MPs that infiltrate the subretinal space following RD are associated with the progression of photoreceptor death, particularly through CCL2/CCR2 signaling [4,16]. Thus, it has been demonstrated that *Ccl2*$^{-/-}$ mice display significantly decreased MP infiltration and photoreceptor apoptosis after RD [16]. In primary retinal mixed cultures, CCL2 also induces photoreceptor loss but its toxicity is abolished through immunodepleting MPs from the culture, suggesting that CCL2 mediates photoreceptor death via activation and recruitment of MPs after RD [16]. In that study, CCL2 was significantly upregulated in the vitreous from RD eyes. Although it did not show any association with the clinical features, it was significantly correlated with all other proteins and might therefore play an important role in the cytokine network in RD. In contrast, we found that CXCL10 expression was strongly correlated to the duration of detachment. CXCL10 is a chemokine of the CXC family which is produced by a variety of cells such as epithelial cells, endothelial cells, fibroblasts, monocytes and neutrophils in response to IFN-γ [34]. It has been shown to exacerbate inflammation and cause significant tissue damage mainly by recruiting activated Th1 lymphocytes to the site of injury [35–37]. Thus, our findings, together with the upregulation of IFN-γ, suggest that the adaptative immune system and Th-1 cells may also contribute to photoreceptor loss following RD.

Consistent with the literature, we found that extent of detachment was associated with the levels of CXCL10 and CCL11, another chemoattractant for granulocytes and

macrophages [12,14,15,38,39]. Furthermore, we observed that both chemokines were correlated with preoperative PVR grade and aqueous flare values, two predictive factors for later PVR development [26,27,40]. These findings are concordant with those of Ricker et al. which reported that both CXCL10 and CCL11 levels were higher in patients who developed postoperative PVR [10]. In addition, several immunohistochemical studies have demonstrated the presence of macrophages and lymphocytes in PVR membranes, further confirming the crucial role of inflammation in the PVR process [19–22,41].

Interestingly, eyes with grade B or C PVR exhibited higher flare values than those without significant PVR, which supports our previous speculation that aqueous flare measurement may be adequate to predict the risk of developing postoperative PVR [26]. Taken together, our results suggest that the upregulation of CXCL10 and CCL11 may predispose to PVR redetachment.

Finally, it should be noted that inflammation is not always detrimental but may also help maintain tissue homeostasis. Thus, the levels of IL-1ra, an inhibitor of the pro-inflammatory effect of IL-1ß, were found to increase with the severity of the disorder. This is consistent with Kiang et al.'s study in which IL-2 and IL-10, two other anti-inflammatory cytokines, were correlated to the extent and duration of detachment [14]. Similarly, Okunuki et al. demonstrated that microglial cells may limit retinal damage and prevent photoreceptor death at 24 h post-RD by controlling macrophage infiltration and by removing potentially damaging cell debris [42].

We acknowledge several limitations to the present study. First, because of our low sample size, we did not address the integrity of the photoreceptor layers on SD-OCT and PVR redetachment, two features that would have been particularly useful to consider in our correlation analysis. Moreover, we did not perform a multivariate regression model that

specifically took into account the most relevant clinical factors. Further studies are required to separate the relationships between variables and to confirm that these factors are independent predictors of cytokine and chemokine concentrations.

In summary, our study confirms that RD causes a marked inflammatory response with a complex cytokine network that may contribute to photoreceptor degeneration and PVR development. We identified proteins specifically associated with several clinical features, including duration and extent of detachment, PVR grade and aqueous flare. Of particular interest is the chemokine CXCL10 which was correlated with all these parameters, suggesting that T-cells play a major role in the pathogenesis of RD. It might represent a potential therapeutic target for improving the anatomical and functional outcomes of RD surgery.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest

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Table 1. Baseline characteristics of patients who underwent vitrectomy for retinal detachment (RD group) and macular hole or vitreomacular traction

	RD group	Control group	P-value
Number of eyes/patients, n	41	33	-
Age, years (mean ± SD)	62.4 ± 12.4	72.2 ± 9.1	<0.001[a]
Male, n(%)	27(65.9)	7(21.2)	<0.001[b]
Lens status			0.031[b]
- *Phakic eyes, n(%)*	17(41.5)	22(66.7)	
- *Pseudophakic eyes, n(%)*	24(58.5)	11(33.3)	
Axial length, mm (mean ± SD)	24.7 ± 1.4	24.3 ± 2.4	0.477[a]
Preoperative BCVA, logMAR (mean ± SD)	1.2 ± 0.9	-	-
Duration of RD, days (mean ± SD)	7.7 ± 7.3	-	-
Extent of RD, quadrants (mean ± SD)	2.1 ± 0.8	-	-
Macular status		-	-
- *Macula on, n(%)*	14(34.1)		
- *Macula off, n(%)*	27(65.9)		
Number of breaks, *n(%)*	1.6 ± 1.0	-	-
Preoperative PVR		-	-
- *None, n(%)*	5(12.2)		
- *Grade A, n(%)*	21(51.2)		
- *Grade B, n(%)*	14(34.2)		
- *Grade C, n(%)*	1(2.4)		
Preoperative aqueous flare values, pc/ms	18.4 ± 19.5	-	-

(mean ± SD)			

SD = standard deviation, BCVA = best-corrected visual acuity, logMAR = logarithm of the minimum angle of resolution, RD = retinal detachment, PVR = proliferative vitreoretinopathy, pc/ms = photon counts per millisecond.

[a]Student *t*-test

[b]Chi-square test

Table 2. Vitreous concentrations of inflammatory mediators from 31 patients with macular hole and 41 patients with retinal detachment

Inflammatory mediators	MH group	RD group	p^a
	Vitreous concentration, pg/ml (mean ± SD)		
Cytokines			
- *IL-1ra*	29.0 ± 99.3	134.8 ± 180.3	**0.006**
- *IL-6*	1.0 ± 2.1	103.0 ± 161.2	**0.003**
- *IL-7*	62.8 ± 110.9	183.5 ± 223.7	**0.002**
- *IL-8*	11.8 ± 15.2	97.5 ± 121.2	**0.004**
- *IL-10*	2.9 ± 4.0	2.0 ± 3.9	0.756
- *IL-13*	1.4 ± 1.4	0.8 ± 1.2	0.987
- *IFN-γ*	1.8 ± 2.3	18.7 ± 13.5	**<0.001**
Chemokines			
- *CCL2*	538.7 ± 327.6	3402.9 ± 2229.7	**<0.001**
- *CCL3*	0.2 ± 0.4	1.9 ± 2.1	**<0.001**
- *CCL4*	1.2 ± 1.6	10.5 ± 11.4	**<0.001**
- *CXCL10*	398.6 ± 382.6	1377.8 ± 2469.8	**0.007**
- *CCL11*	1.3 ± 1.7	3.2 ± 2.9	**0.021**
Growth factors			
- *GCSF*	2.5 ± 8.5	36.6 ± 62.8	**0.014**
- *VEGF*	523.9 ± 215.1	567.0 ± 223.9	0.323

MH = macular hole, RD = retinal detachment, SD = standard deviation, IL = interleukin, IL-1ra = IL-1 receptor antagonist, interferon IFN-γ = interferon-γ, CCL = chemokine C-C motif ligand,

CXCL = chemokine C-X-C motif ligand, GCSF = granulocyte colony-stimulating factor, VEGF = vascular endothelial growth factor

[a] **Means comparisons after post-hoc adjustment for age, lens status, sex and axial length in each group (based on propensity scores analyses)**

Table 3. Pearson's correlation coefficients (R) between upregulated cytokines in the vitreous from eyes with retinal detachment

	IL-1ra	IL-6	IL-7	IL-8	CCL11	G-CSF	IFN-γ	CXCL10	CCL2	CCL3	CCL4
IL-1ra	1.000	-	-	-	-	-	-	-	-	-	-
IL-6	**0.450**	1.000	-	-	-	-	-	-	-	-	-
p	***0.003***										
IL-7	**0.439**	0.305	1.000	-	-	-	-	-	-	-	-
p	***0.004***	*0.052*									
IL-8	0.256	0.251	0.056	1.000	-	-	-	-	-	-	-
p	*0.107*	*0.113*	*0.729*								
CCL11	**0.325**	**0.523**	0.123	0.041	1.000	-	-	-	-	-	-
p	***0.038***	***<0.001***	*0.442*	*0.799*							
G-CSF	**0.574**	**0.753**	0.197	0.104	**0.490**	1.000	-	-	-	-	-
p	***<0.001***	***<0.001***	*0.216*	*0.518*	***0.001***						
IFN-γ	**0.486**	**0.578**	**0.367**	**0.503**	**0.386**	**0.514**	1.000	-	-	-	-

p	***0.001***	***<0.001***	***0.018***	***<0.001***	***0.013***	***<0.001***					
CXCL10	**0.870**	**0.453**	**0.461**	0.094	**0.369**	**0.636**	**0.435**	1.000	-	-	-
p	***<0.001***	***0.003***	***0.002***	*0.561*	***0.018***	***<0.001***	***0.005***				
CCL2	**0.430**	**0.493**	**0.342**	**0.395**	**0.361**	**0.400**	**0.954**	**0.398**	1.000	-	-
p	***0.005***	***0.001***	***0.029***	***0.011***	***0.020***	***0.001***	***<0.001***	***0.001***			
CCL3	**0.791**	**0.510**	**0.475**	**0.416**	0.275	**0.626**	**0.720**	**0.754**	**0.648**	1.000	-
p	***<0.001***	***0.001***	***0.002***	***0.007***	*0.082*	***<0.001***	***<0.001***	***<0.001***	***<0.001***		
CCL4	**0.345**	**0.341**	0.250	**0.478**	0.086	**0.434**	**0.758**	0.154	**0.671**	**0.693**	1.000
p	***0.027***	***0.029***	*0.115*	***0.002***	*0.593*	***0.005***	***<0.001***	*0.3376*	***<0.001***	***<0.001***	

Table 4. Pearson's correlation coefficients (R) between intravitreal cytokines and clinical features

	Cytokines										
	IL-1ra	**IL-6**	**IL-7**	**IL-8**	**CCL11**	**G-CSF**	**IFN-γ**	**CXCL10**	**CCL2**	**CCL3**	**CCL4**
Axial length *(mm)*	-0.117	-0.069	-0.210	-0.236	-0.001	-0.005	-0.185	0.009	-0.163	-0.123	-0.239
p	*0.479*	*0.678*	*0.199*	*0.148*	*0.996*	*0.977*	*0.260*	*0.958*	*0.322*	*0.455*	*0.143*
Flare values *(pc/ms)*	0.175	0.086	0.055	-0.100	**0.563**	0.261	0.150	**0.336**	0.132	0.177	-0.041
p	*0.295*	*0.606*	*0.744*	*0.551*	***<0.001***	*0.113*	*0.370*	***0.039***	*0.430*	*0.288*	*0.807*
Duration of RD *(days)*	**0.369**	0.206	0.215	-0.050	0.227	0.254	-0.049	**0.451**	-0.048	0.269	-0.193
p	***0.019***	*0.201*	*0.183*	*0.760*	*0.160*	*0.114*	*0.762*	***0.004***	*0.768*	*0.094*	*0.233*
Extent of RD *(quadrants)*	**0.451**	0.128	0.116	0.038	**0.320**	**0.324**	-0.017	**0.439**	-0.071	0.228	-0.116
p	***0.003***	*0.427*	*0.471*	*0.814*	***0.041***	***0.039***	*0.915*	***0.004***	*0.657*	*0.152*	*0.471*
Number of breaks	0.109	0.158	0.118	0.005	0.101	0.079	0.051	0.222	0.081	0.052	-0.095
p	*0.497*	*0.325*	*0.461*	*0.976*	*0.528*	*0.624*	*0.752*	*0.164*	*0.616*	*0.748*	*0.555*
Preoperative BCVA *(logMAR)*	0.285	0.111	0.107	0.130	0.065	0.001	-0.023	0.179	-0.086	0.027	-0.166
p	*0.071*	*0.488*	*0.504*	*0.419*	*0.689*	*0.996*	*0.888*	*0.262*	*0.592*	*0.865*	*0.301*

RD = retinal detachment, pc/ms = photon counts per millisecond, BCVA = best-corrected visual acuity, logMAR = logarithm of the minimum angle of resolution

Table 5. Comparison of CXCL10 and CCL11 intravitreal levels and flare values according to preoperative proliferative vitreoretinopathy

	Preoperative PVR		*p*
	0 or grade A	Grade B or C	
Cytokine level, pg/ml (mean ± SD)			
- *CCL11*	2.3 ± 2.5	4.6 ± 3.0	***0.019***[a]
- *CXCL10*	738.0 ± 517.2	2486.9 ± 3856.4	***0.005***[a]
Preoperative flare values, pc/ms (mean ± SD)	8.9 ± 2.6	33.0 ± 24.8	***< 0.001***[a]

[a] Mann-Whitney-Wilcoxon test

SD = standard deviation, PVR = proliferative vitreoretinopathy, pc/ms = photon counts per milliseconds