

RESEARCH ARTICLE

Biomarkers for PVR in rhegmatogenous retinal detachment

Souska Zandi¹, Isabel B. Pfister¹, Peter G. Traine¹, Christoph Tappeiner²,
Alain Despont³, Robert Rieben³, Magdalena Skowronska¹, Justus G. Garweg^{1,4*}

1 Swiss Eye Institute and Clinic for Vitreoretinal Diseases, Berner Augenklinik am Lindenhof-Spital, Bern, Switzerland, **2** Department of Ophthalmology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, **3** Department for Biomedical Research, University of Bern, Bern, Switzerland, **4** University of Bern, Bern, Switzerland

* garweg@swiss-eye-institute.com



Abstract

Purpose

Various profibrotic and proinflammatory cytokines have been found upregulated in uncomplicated primary retinal detachment (pRD), but without providing a uniform picture. Here, we compare the cyto- and chemokine profiles in pRD with and without proliferative vitreoretinopathy (PVR) in an attempt to unravel relevant differences not in single cytokines, but in the cytokine profiles at diagnosis.

Methods

Undiluted vitreous fluid (VF) was obtained at the beginning of surgery from 174 eyes with pRD without relevant PVR (maximally grade B; group 1; $n = 81$) and with moderate or advanced PVR requiring a gas tamponade (group 2; $n = 49$) or silicon oil filling (group 3; $n = 44$). VF of eyes undergoing macular hole (MH) surgery served as controls (group 4; $n = 26$). Forty-three cytokines were quantified in parallel using a multiplex cytokine analysis system (Bioplex). For all comparisons we applied Holm's correction to control for multiple comparisons.

Results

44.9% of group 2 eyes presented grade C1 and 55.1% C2-C3, whereas 86.4% of group 3 eyes exhibited a PVR grade of C2-D.

CCL19 was the only cytokine that displayed higher concentrations in the vitreous of eyes with PVR C1 compared to lower PVR grades. Eyes with PVR C2-D showed higher levels of CCL27, CXCL6, IL4, IL16, CXCL10, CCL8, CCL22, MIG/CXCL9, CCL15, CCL19, CCL 23 and CXCL12 compared to controls. Interestingly, no difference of cytokine levels was detected between C1 and C2-D PVR.

Conclusions

CCL19 may represent a potential biomarker for early PVR progression that holds promise for future diagnostic and therapeutic applications.

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Introduction

Cell-signaling mediators, such as cytokines and chemokines are involved in the regulation of inflammatory processes, wound healing and scar formation [1]. In eyes with retinal detachment, elevated levels of a variety of cytokines and growth factors in the vitreous have been reported [2–7].

Retinal detachment (RD) induces cell migration and proliferation as well as the production of extracellular matrix proteins, which in turn lead to the development and contraction of vitreal and periretinal membranes, both hallmarks of proliferative vitreoretinopathy (PVR) [8,9,10]. PVR occurs in up to 10% of rhegmatogenous retinal detachment cases and is the major cause of poor functional outcomes after primarily successful RD surgery [11,12]. Depending on the duration and extent of RD, the accumulation of fibroblasts, collagen, and extracellular matrix components may co-occur with the formation of membranes on the vitreous and the interfaces of the entire retina, including the still attached parts. Previous studies found associations between PVR and significantly increased concentrations of certain pro-inflammatory cytokines and growth factors in the vitreous [13–19]. The majority of which did not exhibit any differences in their clinical relevance, except for IL-1, IL-6, IL-8, IL-10, TNF-alpha, IL1-beta, IFN gamma, ICAM-1, PDGF, MIF (macrophage inhibitory factor) and the chemokine ligands CCL2, CCL11, CCL17, CCL18, CCL19, CCL22, CXCL8, CXCL9 and CXCL10 [17,18,20].

The aim of this study was to not only focus on single cytokines in the vitreous fluid (VF) when comparing between cases with and without relevant PVR, but to provide a much broader comparison of profiles of pro-inflammatory and pro-fibrotic cytokines in the vitreous fluid (VF) which should facilitate an improved differentiation of their relative importance with respect to the pathophysiological process of PVR.

Patients and methods

Patients

The investigation was designed as a prospective study involving consecutive patients undergoing pars plana vitrectomy for the treatment of primary retinal detachment with and without PVR and subsequent gas or silicon oil filling. All surgeries had been performed by the same surgeon at the Berner Augenklinik am Lindenhofspital, Bern, Switzerland. Patients with systemic or ocular comorbidities that may potentially influence ocular cytokine levels were excluded, i.e. patients with diabetes mellitus, known rheumatic and autoimmune diseases, systemic treatments involving corticosteroids or immunomodulatory drugs, vitreous hemorrhaging, uveitis, glaucoma, or any concomitant retinal pathology, or who had undergone intraocular surgery or treatment within six months of the RD diagnosis. If both eyes were affected, only the first operated eye was included. The exclusion criteria apply also to the control group.

The study was approved by the Ethics committee of the University of Bern (KEK no. 152/08), and is fully compliant with the tenets of the Declaration of Helsinki. Each participant provided their informed written consent to the use of their biological materials and clinical data.

PVR grading and patient grouping

Following recommendations from the “Retina Society Terminology Committee (1983)” [21], we classified patients based on PVR severity into four stages: A (minimal), B, C, and D (massive). For the purpose of this study, we considered the risk of developing postoperative PVR to be similar in pRD patients without PVR and pRD patients with low PVR severity (grades A or

B) (denoted collectively as group 1, $n = 81$). On the basis of this classification system, PVR grade C was further subcategorized into C1–C3, with the numerals 1–3 referring to the number of quadrants with visible PVR membrane formation. If all 4 quadrants were affected, the severity was defined as grade D. Since accurate grading in advanced PVR may be difficult, wide field images (Optos TX200, Optos Inc, Dunfermline, Scotland) were obtained from all eyes prior to surgery. To examine postoperative PVR risk, patients with advanced PVR were grouped according to the intraoperative decision regarding the type of tamponade (Fig 1): eyes receiving an SF₆ gas tamponade were categorized as group 2 ($n = 49$, those in need of a silicone oil tamponade are group 3 ($n = 44$), while those undergoing macular hole (MH) surgery with SF₆ gas tamponade served as control (group 4; $n = 26$).

Handling of vitreous fluid samples

Approximately 500 microliters of undiluted VF were collected at the beginning of pars plana vitrectomy. After harvesting, the VF initially stored at -20°C and moved to -80°C after

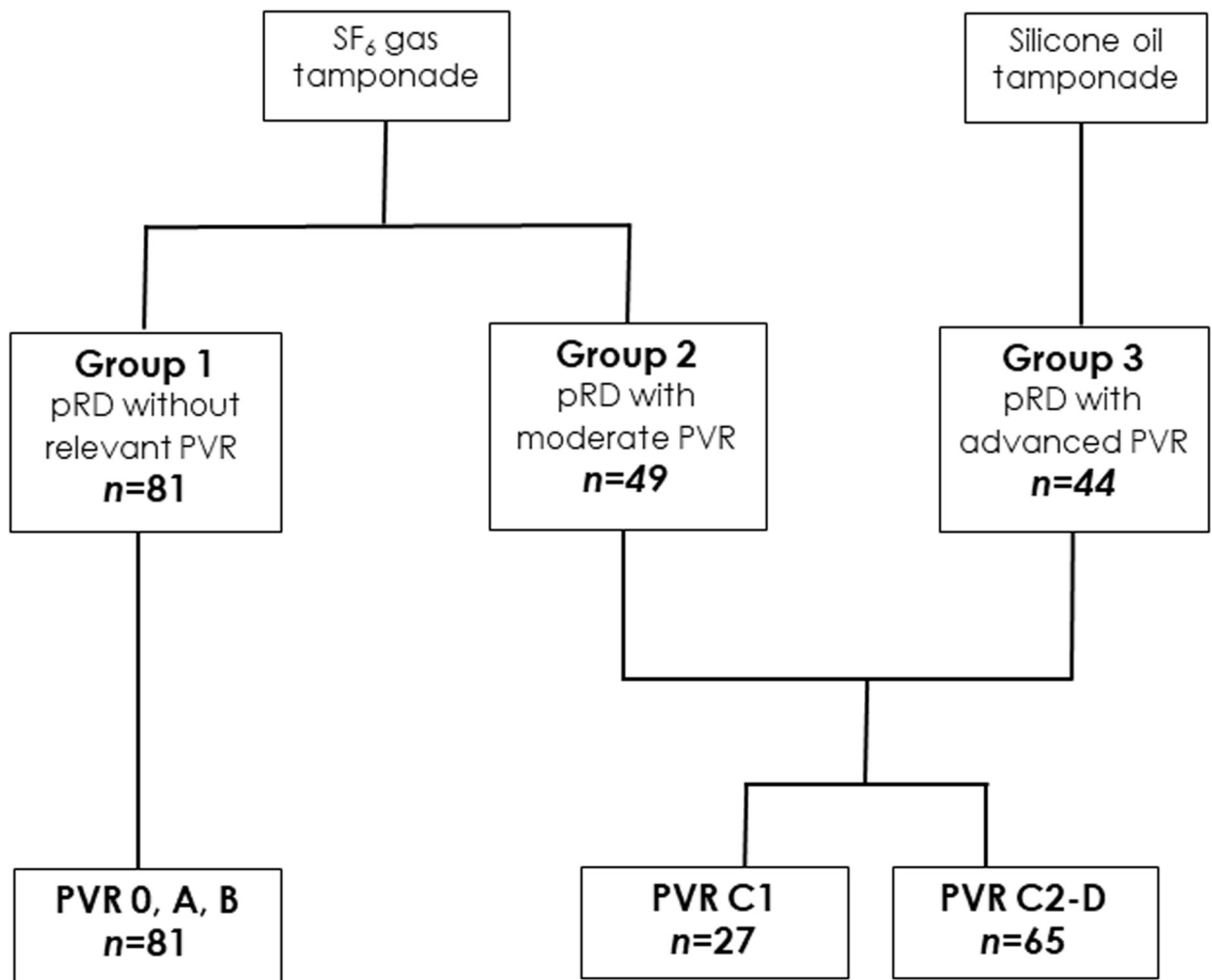


Fig 1. Schematic diagram of groups and PVR grades. Group 1 eyes showed a maximal PVR grade A or B (no relevant vascular wrinkling), whereas 44.9% of group 2 presented a PVR grade C1 and in 55.1% grades C2 or C3. However, 86.4% of eyes in group 3 exhibited a PVR grade of C2-D and required silicone oil tamponade. Therefore, we labeled these groups as “without relevant PVR” (group 1), moderate (group 2) and advanced PVR (group 3).

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maximally 2 months where it remained until the final analysis which was performed on all samples simultaneously.

Cytokine analyses

The samples were analyzed using a multiplex system (Bio-Plex 100 array reader with Bio-Plex Manager software version 6.1; Bio-Rad, Hercules, CA, USA). With this highly sensitive technique, multiple analytes can be detected in parallel using a single small volume sample. We quantified the concentrations of 43 cytokines in each vitreous sample (Tables 1–6). All analytic procedures were performed following the manufacturer's instructions. In short, magnetic microspheres, tagged with a fluorescent label were coupled to specific capture antibodies and mixed with samples containing unknown cytokine quantities before introducing biotinylated detection antibodies and Streptavidin R-Phycoerythrin. The mixture was then analyzed by flow cytometry. The instrument's two lasers identify the microsphere type and quantify the amount of bound antigen. On each test plate we ran a duplicate concentration standard in parallel for each cytokine. The measurements were performed in a blinded manner by a laboratory technician who was experienced in the execution of this technique.

Statistical analyses

By performing a Shapiro-Wilk test we found that our data were not normally distributed. As a result, we employed a non-parametric Mann-Whitney U test for the inter-group and intra-group comparisons. A p-value of <0.05 was considered to be significant. Since multiple comparisons increase the risk of introducing a Type-I error, we applied the sequentially rejective Bonferroni correction (Holm's correction) to control for this type of error without introducing additional Type II errors [22,23]. This means that the p-value must be divided by the number of tests run in parallel, resulting in an adjusted level of statistical significance of $p = 0.05/43 = 0.00116$. All statistical analyses were performed using R (package FSA, software version 3.4.0).

Results

Patients

The eyes of 174 consecutive patients admitted to our clinic for primary retinal detachment met the inclusion criteria (group 1 $n = 81$; group 2 $n = 49$; group 3 $n = 44$). Thereof, 92 eyes were phakic and 82 eyes were pseudophakic (group 1: $n = 40$, group 2: $n = 17$, group 3: $n = 25$). In the control group (group 4), 26 patients with macular hole underwent pars plana vitrectomy, all of which were phakic before surgery. Since we recently showed that the lens status does not significantly influence the cytokine profiles in the VF phakic and pseudophakic eyes in each group were pooled [24].

The mean age in group 4 was higher than in groups 1 and 2 while similar to group 3 (group 4 (control): 67.5 ± 8.2 years; group 1: 61.6 ± 14.3 years; group 2: 61.9 ± 9.6 ; group 3: 68.5 ± 14.1). While the control group (group 4) and group 2 were predominantly female (group 4: 19.2% males, 80.8% females, group 2: 38.8% males, 60.2% females) group 1 and 3 contained male majorities (group 1: 65.4% males; group 3: 59.1% males). However there were no significant differences in gender between the RD groups.

Comparison of cytokine profiles in macular hole and pRD

Compared to the control group, pRD patients both with or without PVR displayed significantly higher levels of all chemo- and cytokines in the VF, except for TGF beta-1 and -2, even after correction for multiple comparisons (Fig 2).

Table 1. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment without PVR (pRD) and in eyes with primary retinal detachment with moderate PVR treated with SF₆ gas tamponade.

Cytokine	pRD		moderate PVR		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	2381.8	5022.1	1994.3	2675.1	<i>p</i> = 0.333
CXCL13	1.9	2.8	2.3	2.7	<i>p</i> = 0.01795
CCL27	10.6	41.0	11.5	24.2	<i>p</i> = 0.08551
CXCL5	164.7	195.1	189.8	184.3	<i>p</i> = 0.2683
CCL11	13.1	16.1	16.4	17.2	<i>p</i> = 0.04332
CCL24	21.4	22.2	22.1	14.0	<i>p</i> = 0.1448
CCL26	9.9	14.5	12.1	15.5	<i>p</i> = 0.03833
CX3CL1	67.7	70.9	88.9	91.0	<i>p</i> = 0.05434
CXCL6	2.3	3.8	3.5	4.8	<i>p</i> = 0.08683
GM-CSF	45.9	18.0	49.5	21.2	<i>p</i> = 0.3153
CXCL1	66.4	64.0	80.3	62.0	<i>p</i> = 0.03006
CXCL2	22.5	51.1	46.6	96.9	<i>p</i> = 0.01459
CCL1	35.5	59.3	49.0	68.1	<i>p</i> = 0.02713
IFN- γ	8.7	11.9	11.8	14.3	<i>p</i> = 0.1089
IL-1 β	1.5	2.2	2.3	3.1	<i>p</i> = 0.02623
IL-2	1.5	1.8	1.8	1.3	<i>p</i> = 0.02031
IL-4	3.0	4.8	4.8	5.7	<i>p</i> = 0.03141
IL-6	136.0	349.1	418.3	1393.3	<i>p</i> = 0.0312
IL-8/CXCL8	36.5	51.2	46.1	51.9	<i>p</i> = 0.04618
IL-10	7.2	5.4	8.6	4.6	<i>p</i> = 0.0295
IL-16	57.6	45.1	92.3	110.0	<i>p</i> = 0.01556
CXCL10	350.3	1529.3	303.0	628.0	<i>p</i> = 0.07788
CXCL11	5.0	8.1	5.7	7.2	<i>p</i> = 0.1627
CCL2	1602.8	1597.1	1892.5	1415.4	<i>p</i> = 0.04438
CCL8	12.4	30.6	13.2	18.7	<i>p</i> = 0.03119
CCL7	21.8	26.0	29.3	32.4	<i>p</i> = 0.08307
CCL13	2.3	2.4	3.1	3.3	<i>p</i> = 0.01818
CCL22	14.0	17.3	15.1	11.6	<i>p</i> = 0.2365
MIF	101278.2	99480.3	113452.4	105774.0	<i>p</i> = 0.5058
MIG/CXCL9	304.1	2254.6	138.7	666.4	<i>p</i> = 0.09597
CCL3	3.1	2.7	4.0	3.2	<i>p</i> = 0.02018
CCL15	712.1	600.2	930.3	1049.4	<i>p</i> = 0.3806
CCL20	10.9	14.9	17.4	25.6	<i>p</i> = 0.0232
CCL19	38.1	62.8	47.4	48.9	<i>p</i> = 0.002001
CCL23	17.4	19.9	21.6	24.6	<i>p</i> = 0.1465
CXCL16	812.5	313.6	911.5	312.8	<i>p</i> = 0.0816
CXCL12	169.6	233.9	194.8	137.4	<i>p</i> = 0.003913
CCL17	6.4	19.2	8.4	15.1	<i>p</i> = 0.0775
CCL25	384.9	498.9	464.4	568.1	<i>p</i> = 0.08286
TNF- α	13.3	17.8	15.9	13.8	<i>p</i> = 0.04564
TGF- β 1	100.1	215.5	34.5	74.2	<i>p</i> = 0.08435
TGF- β 2	1243.6	823.9	871.0	673.7	<i>p</i> = 0.01038
TGF- β 3	12.3	26.4	6.1	12.6	<i>p</i> = 0.2018

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Table 2. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment without PVR (pRD) and in eyes with primary retinal detachment with advanced PVR requiring silicone oil tamponade.

Cytokine	pRD		advanced PVR		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	2381.8	5022.1	4226.2	7403.1	<i>p</i> = 0.003323
CXCL13	1.9	2.8	4.1	8.0	<i>p</i> = 0.001729
CCL27	10.6	41.0	37.3	102.3	<i>p</i> = 1.27E-06
CXCL5	164.7	195.1	178.7	195.4	<i>p</i> = 0.7854
CCL11	13.1	16.1	20.8	22.2	<i>p</i> = 0.007636
CCL24	21.4	22.2	37.1	45.8	<i>p</i> = 0.00504
CCL26	9.9	14.5	17.3	19.2	<i>p</i> = 0.001253
CX3CL1	67.7	70.9	98.2	92.3	<i>p</i> = 0.009529
CXCL6	2.3	3.8	6.9	8.5	<i>p</i> = 0.0002024
GM-CSF	45.9	18.0	52.8	19.2	<i>p</i> = 0.04683
CXCL1	66.4	64.0	83.7	59.9	<i>p</i> = 0.01489
CXCL2	22.5	51.1	71.1	112.7	<i>p</i> = 0.004821
CCL1	35.5	59.3	65.6	97.6	<i>p</i> = 0.02381
IFN- γ	8.7	11.9	17.2	18.2	<i>p</i> = 0.00204
IL-1 β	1.5	2.2	2.3	3.5	<i>p</i> = 0.08084
IL-2	1.5	1.8	2.0	1.5	<i>p</i> = 0.01801
IL-4	3.0	4.8	6.5	6.6	<i>p</i> = 0.0002875
IL-6	136.0	349.1	1348.3	6939.1	<i>p</i> = 0.007979
IL-8/CXCL8	36.5	51.2	55.2	59.7	<i>p</i> = 0.007763
IL-10	7.2	5.4	10.0	6.1	<i>p</i> = 0.006387
IL-16	57.6	45.1	119.4	113.4	<i>p</i> = 0.0002374
CXCL10	350.3	1529.3	418.4	590.2	<i>p</i> = 3.39E-05
CXCL11	5.0	8.1	7.9	10.0	<i>p</i> = 0.03648
CCL2	1602.8	1597.1	1815.8	1941.9	<i>p</i> = 0.7604
CCL8	12.4	30.6	19.6	24.8	<i>p</i> = 0.0003082
CCL7	21.8	26.0	35.9	35.3	<i>p</i> = 0.01288
CCL13	2.3	2.4	3.9	3.8	<i>p</i> = 0.01031
CCL22	14.0	17.3	27.8	25.4	<i>p</i> = 0.0001539
MIF	101278.2	99480.3	138533.6	128551.1	<i>p</i> = 0.07621
MIG/CXCL9	304.1	2254.6	470.3	1452.7	<i>p</i> = 1.05E-05
CCL3	3.1	2.7	4.6	4.3	<i>p</i> = 0.007524
CCL15	712.1	600.2	1901.6	2228.4	<i>p</i> = 0.0001252
CCL20	10.9	14.9	20.3	35.9	<i>p</i> = 0.00208
CCL19	38.1	62.8	93.7	143.2	<i>p</i> = 4.26E-05
CCL23	17.4	19.9	37.9	44.1	<i>p</i> = 1.27E-05
CXCL16	812.5	313.6	996.9	356.6	<i>p</i> = 0.003985
CXCL12	169.6	233.9	330.4	335.7	<i>p</i> = 2.07E-05
CCL17	6.4	19.2	14.0	26.8	<i>p</i> = 0.006175
CCL25	384.9	498.9	613.3	628.8	<i>p</i> = 0.005957
TNF- α	13.3	17.8	19.1	15.7	<i>p</i> = 0.005545
TGF- β 1	100.1	215.5	118.6	214.8	<i>p</i> = 0.5693
TGF- β 2	1243.6	823.9	1221.0	1213.8	<i>p</i> = 0.2869
TGF- β 3	12.3	26.4	15.0	24.7	<i>p</i> = 0.7028

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Table 3. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment with moderate PVR treated with SF₆ gas tamponade and in eyes with primary retinal detachment with advanced PVR requiring silicone oil tamponade.

Cytokine	moderate PVR		advanced PVR		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	1994.3	2675.1	4226.2	7403.1	<i>p</i> = 0.07548
CXCL13	2.3	2.7	4.1	8.0	<i>p</i> = 0.1684
CCL27	11.5	24.2	37.3	102.3	<i>p</i> = 0.003019
CXCL5	189.8	184.3	178.7	195.4	<i>p</i> = 0.5609
CCL11	16.4	17.2	20.8	22.2	<i>p</i> = 0.453
CCL24	22.1	14.0	37.1	45.8	<i>p</i> = 0.166
CCL26	12.1	15.5	17.3	19.2	<i>p</i> = 0.2041
CX3CL1	88.9	91.0	98.2	92.3	<i>p</i> = 0.4059
CXCL6	3.5	4.8	6.9	8.5	<i>p</i> = 0.03418
GM-CSF	49.5	21.2	52.8	19.2	<i>p</i> = 0.5081
CXCL1	80.3	62.0	83.7	59.9	<i>p</i> = 0.6035
CXCL2	46.6	96.9	71.1	112.7	<i>p</i> = 0.6324
CCL1	49.0	68.1	65.6	97.6	<i>p</i> = 0.8024
IFN- γ	11.8	14.3	17.2	18.2	<i>p</i> = 0.1102
IL-1 β	2.3	3.1	2.3	3.5	<i>p</i> = 0.7817
IL-2	1.8	1.3	2.0	1.5	<i>p</i> = 0.7002
IL-4	4.8	5.7	6.5	6.6	<i>p</i> = 0.1649
IL-6	418.3	1393.3	1348.3	6939.1	<i>p</i> = 0.4236
IL-8/CXCL8	46.1	51.9	55.2	59.7	<i>p</i> = 0.4886
IL-10	8.6	4.6	10.0	6.1	<i>p</i> = 0.3951
IL-16	92.3	110.0	119.4	113.4	<i>p</i> = 0.1934
CXCL10	303.0	628.0	418.4	590.2	<i>p</i> = 0.01602
CXCL11	5.7	7.2	7.9	10.0	<i>p</i> = 0.4862
CCL2	1892.5	1415.4	1815.8	1941.9	<i>p</i> = 0.2015
CCL8	13.2	18.7	19.6	24.8	<i>p</i> = 0.1095
CCL7	29.3	32.4	35.9	35.3	<i>p</i> = 0.3634
CCL13	3.1	3.3	3.9	3.8	<i>p</i> = 0.4393
CCL22	15.1	11.6	27.8	25.4	<i>p</i> = 0.008345
MIF	113452.4	105774.0	138533.6	128551.1	<i>p</i> = 0.2813
MIG/CXCL9	138.7	666.4	470.3	1452.7	<i>p</i> = 0.002084
CCL3	4.0	3.2	4.6	4.3	<i>p</i> = 0.7033
CCL15	930.3	1049.4	1901.6	2228.4	<i>p</i> = 0.007758
CCL20	17.4	25.6	20.3	35.9	<i>p</i> = 0.3227
CCL19	47.4	48.9	93.7	143.2	<i>p</i> = 0.2001
CCL23	21.6	24.6	37.9	44.1	<i>p</i> = 0.0008617
CXCL16	911.5	312.8	996.9	356.6	<i>p</i> = 0.1895
CXCL12	194.8	137.4	330.4	335.7	<i>p</i> = 0.05835
CCL17	8.4	15.1	14.0	26.8	<i>p</i> = 0.3305
CCL25	464.4	568.1	613.3	628.8	<i>p</i> = 0.2154
TNF- α	15.9	13.8	19.1	15.7	<i>p</i> = 0.338
TGF- β 1	34.5	74.2	118.6	214.8	<i>p</i> = 0.0453
TGF- β 2	871.0	673.7	1221.0	1213.8	<i>p</i> = 0.1988
TGF- β 3	1994.3	2675.1	15.0	24.7	<i>p</i> = 0.1831

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Table 4. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment with PVR grade B or less and in eyes with PVR C1.

Cytokine	PVR grade B or less		PVR grade C1		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	2381.8	5022.1	2617.5	3303.7	<i>p</i> = 0.141
CXCL13	1.9	2.8	2.2	2.2	<i>p</i> = 0.01794
CCL27	10.6	41.0	11.0	21.0	<i>p</i> = 0.1178
CXCL5	164.7	195.1	211.5	196.1	<i>p</i> = 0.1571
CCL11	13.1	16.1	20.1	20.0	<i>p</i> = 0.02337
CCL24	21.4	22.2	24.3	16.1	<i>p</i> = 0.1088
CCL26	9.9	14.5	15.5	19.3	<i>p</i> = 0.02906
CX3CL1	67.7	70.9	95.3	93.8	<i>p</i> = 0.05406
CXCL6	2.3	3.8	3.7	5.7	<i>p</i> = 0.1644
GM-CSF	45.9	18.0	51.1	20.6	<i>p</i> = 0.2547
CXCL1	66.4	64.0	84.9	63.9	<i>p</i> = 0.02091
CXCL2	22.5	51.1	52.9	119.0	<i>p</i> = 0.09327
CCL1	35.5	59.3	60.2	79.5	<i>p</i> = 0.02523
IFN- γ	8.7	11.9	12.6	15.2	<i>p</i> = 0.1052
IL-1 β	1.5	2.2	1.8	1.8	<i>p</i> = 0.2038
IL-2	1.5	1.8	1.8	1.3	<i>p</i> = 0.05152
IL-4	3.0	4.8	5.1	6.2	<i>p</i> = 0.0411
IL-6	136.0	349.1	572.6	1834.9	<i>p</i> = 0.1652
IL-8/CXCL8	36.5	51.2	43.5	43.5	<i>p</i> = 0.09129
IL-10	7.2	5.4	9.4	4.6	<i>p</i> = 0.01694
IL-16	57.6	45.1	73.6	45.4	<i>p</i> = 0.07492
CXCL10	350.3	1529.3	387.8	803.8	<i>p</i> = 0.1709
CXCL11	5.0	8.1	7.1	8.6	<i>p</i> = 0.07255
CCL2	1602.8	1597.1	1951.5	1681.5	<i>p</i> = 0.0596
CCL8	12.4	30.6	14.9	23.1	<i>p</i> = 0.06931
CCL7	21.8	26.0	34.0	39.3	<i>p</i> = 0.1071
CCL13	2.3	2.4	3.6	3.8	<i>p</i> = 0.01105
CCL22	14.0	17.3	18.2	21.1	<i>p</i> = 0.3609
MIF	101278.2	99480.3	124323.2	117135.7	<i>p</i> = 0.4085
MIG/CXCL9	304.1	2254.6	222.7	895.7	<i>p</i> = 0.07377
CCL3	3.1	2.7	4.5	4.0	<i>p</i> = 0.03388
CCL15	712.1	600.2	1166.5	1704.2	<i>p</i> = 0.4289
CCL20	10.9	14.9	15.2	17.0	<i>p</i> = 0.05405
CCL19	38.1	62.8	55.8	55.5	<i>p</i> = 0.0009894
CCL23	17.4	19.9	22.9	27.8	<i>p</i> = 0.1199
CXCL16	812.5	313.6	994.4	339.3	<i>p</i> = 0.02015
CXCL12	169.6	233.9	211.9	156.9	<i>p</i> = 0.006792
CCL17	6.4	19.2	10.4	18.5	<i>p</i> = 0.06664
CCL25	384.9	498.9	571.1	684.8	<i>p</i> = 0.03157
TNF- α	13.3	17.8	18.6	16.2	<i>p</i> = 0.01663
TGF- β 1	100.1	215.5	41.8	134.9	<i>p</i> = 0.06634
TGF- β 2	1243.6	823.9	899.5	677.8	<i>p</i> = 0.06155
TGF- β 3	12.3	26.4	6.2	16.1	<i>p</i> = 0.08247

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Table 5. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment with PVR grade B or less and in eyes with primary retinal detachment and PVR C2-D.

Cytokine	PVR grade B or less		PVR grade C2 or higher		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	2381.8	5022.1	3268.7	6271.7	<i>p</i> = 0.02321
CXCL13	1.9	2.8	3.6	6.9	<i>p</i> = 0.001814
CCL27	10.6	41.0	29.4	86.2	<i>p</i> = 1.75E-05
CXCL5	164.7	195.1	174.5	187.2	<i>p</i> = 0.7461
CCL11	13.1	16.1	18.0	19.9	<i>p</i> = 0.01595
CCL24	21.4	22.2	31.6	38.8	<i>p</i> = 0.009679
CCL26	9.9	14.5	14.4	16.8	<i>p</i> = 0.002285
CX3CL1	67.7	70.9	93.3	91.4	<i>p</i> = 0.0134
CXCL6	2.3	3.8	5.7	7.4	<i>p</i> = 0.0004163
GM-CSF	45.9	18.0	51.7	19.5	<i>p</i> = 0.06252
CXCL1	66.4	64.0	81.1	60.2	<i>p</i> = 0.01964
CXCL2	22.5	51.1	57.3	96.8	<i>p</i> = 0.002432
CCL1	35.5	59.3	56.0	85.9	<i>p</i> = 0.02527
IFN- γ	8.7	11.9	15.4	17.0	<i>p</i> = 0.004325
IL-1 β	1.5	2.2	2.5	3.7	<i>p</i> = 0.01877
IL-2	1.5	1.8	1.9	1.5	<i>p</i> = 0.01031
IL-4	3.0	4.8	5.9	6.2	<i>p</i> = 0.0005886
IL-6	136.0	349.1	986.2	5718.1	<i>p</i> = 0.003728
IL-8/CXCL8	36.5	51.2	52.1	59.8	<i>p</i> = 0.009737
IL-10	7.2	5.4	9.2	5.7	<i>p</i> = 0.01423
IL-16	57.6	45.1	118.1	128.7	<i>p</i> = 0.0002389
CXCL10	350.3	1529.3	345.9	521.2	<i>p</i> = 0.0001969
CXCL11	5.0	8.1	6.7	8.8	<i>p</i> = 0.0757
CCL2	1602.8	1597.1	1832.7	1693.6	<i>p</i> = 0.3683
CCL8	12.4	30.6	16.7	21.7	<i>p</i> = 0.0006981
CCL7	21.8	26.0	32.0	31.8	<i>p</i> = 0.01758
CCL13	2.3	2.4	3.5	3.6	<i>p</i> = 0.01689
CCL22	14.0	17.3	22.4	20.2	<i>p</i> = 0.001134
MIF	101278.2	99480.3	127494.3	118058.1	<i>p</i> = 0.1139
MIG/CXCL9	304.1	2254.6	330.3	1208.3	<i>p</i> = 0.0001447
CCL3	3.1	2.7	4.2	3.7	<i>p</i> = 0.005434
CCL15	712.1	600.2	1502.8	1806.4	<i>p</i> = 0.0007773
CCL20	10.9	14.9	20.3	35.1	<i>p</i> = 0.002397
CCL19	38.1	62.8	75.7	122.2	<i>p</i> = 0.0001397
CCL23	17.4	19.9	32.3	38.8	<i>p</i> = 0.0002163
CXCL16	812.5	313.6	945.8	324.0	<i>p</i> = 0.01275
CXCL12	169.6	233.9	280.8	291.1	<i>p</i> = 4.18E-05
CCL17	6.4	19.2	11.2	22.9	<i>p</i> = 0.01808
CCL25	384.9	498.9	526.9	566.7	<i>p</i> = 0.0152
TNF- α	13.3	17.8	17.0	14.3	<i>p</i> = 0.01748
TGF- β 1	100.1	215.5	88.9	171.9	<i>p</i> = 0.8986
TGF- β 2	1243.6	823.9	1107.2	1078.3	<i>p</i> = 0.08566
TGF- β 3	12.3	26.4	12.1	20.9	<i>p</i> = 0.781

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Table 6. Mean concentrations (pg/ml) and standard deviations (SD) of cytokines in the vitreous of eyes with primary retinal detachment with PVR grade C1, and in eyes with PVR grade C2 or higher.

Cytokine	PVR C1		PVR C2 or higher		Mann-Whitney U test
	Mean (pg/ml)	SD	Mean (pg/ml)	SD	
CCL21	2617.5	3303.7	3268.7	6271.7	<i>p</i> = 0.8537
CXCL13	2.2	2.2	3.6	6.9	<i>p</i> = 0.6189
CCL27	11.0	21.0	29.4	86.2	<i>p</i> = 0.05675
CXCL5	211.5	196.1	174.5	187.2	<i>p</i> = 0.2931
CCL11	20.1	20.0	18.0	19.9	<i>p</i> = 0.665
CCL24	24.3	16.1	31.6	38.8	<i>p</i> = 0.797
CCL26	15.5	19.3	14.4	16.8	<i>p</i> = 0.9795
CX3CL1	95.3	93.8	93.3	91.4	<i>p</i> = 0.9897
CXCL6	3.7	5.7	5.7	7.4	<i>p</i> = 0.1623
GM-CSF	51.1	20.6	51.7	19.5	<i>p</i> = 0.8977
CXCL1	84.9	63.9	81.1	60.2	<i>p</i> = 0.625
CXCL2	52.9	119.0	57.3	96.8	<i>p</i> = 0.6923
CCL1	60.2	79.5	56.0	85.9	<i>p</i> = 0.6037
IFN- γ	12.6	15.2	15.4	17.0	<i>p</i> = 0.5475
IL-1 β	1.8	1.8	2.5	3.7	<i>p</i> = 0.589
IL-2	1.8	1.3	1.9	1.5	<i>p</i> = 0.8705
IL-4	5.1	6.2	5.9	6.2	<i>p</i> = 0.5622
IL-6	572.6	1834.9	986.2	5718.1	<i>p</i> = 0.1674
IL-8/CXCL8	43.5	43.5	52.1	59.8	<i>p</i> = 0.7543
IL-10	9.4	4.6	9.2	5.7	<i>p</i> = 0.6099
IL-16	73.6	45.4	118.1	128.7	<i>p</i> = 0.181
CXCL10	387.8	803.8	345.9	521.2	<i>p</i> = 0.1597
CXCL11	7.1	8.6	6.7	8.8	<i>p</i> = 0.7028
CCL2	1951.5	1681.5	1832.7	1693.6	<i>p</i> = 0.3725
CCL8	14.9	23.1	16.7	21.7	<i>p</i> = 0.3611
CCL7	34.0	39.3	32.0	31.8	<i>p</i> = 0.8067
CCL13	3.6	3.8	3.5	3.6	<i>p</i> = 0.5598
CCL22	18.2	21.1	22.4	20.2	<i>p</i> = 0.153
MIF	124323.2	117135.7	127494.3	118058.1	<i>p</i> = 0.7543
MIG/CXCL9	222.7	895.7	330.3	1208.3	<i>p</i> = 0.2169
CCL3	4.5	4.0	4.2	3.7	<i>p</i> = 0.8807
CCL15	1166.5	1704.2	1502.8	1806.4	<i>p</i> = 0.081
CCL20	15.2	17.0	20.3	35.1	<i>p</i> = 0.6099
CCL19	55.8	55.5	75.7	122.2	<i>p</i> = 0.7739
CCL23	22.9	27.8	32.3	38.8	<i>p</i> = 0.1042
CXCL16	994.4	339.3	945.8	324.0	<i>p</i> = 0.6464
CXCL12	211.9	156.9	280.8	291.1	<i>p</i> = 0.474
CCL17	10.4	18.5	11.2	22.9	<i>p</i> = 0.9853
CCL25	571.1	684.8	526.9	566.7	<i>p</i> = 0.9044
TNF- α	18.6	16.2	17.0	14.3	<i>p</i> = 0.6099
TGF- β 1	41.8	134.9	88.9	171.9	<i>p</i> = 0.04595
TGF- β 2	899.5	677.8	1107.2	1078.3	<i>p</i> = 0.5229
TGF- β 3	6.2	16.1	12.1	20.9	<i>p</i> = 0.05585

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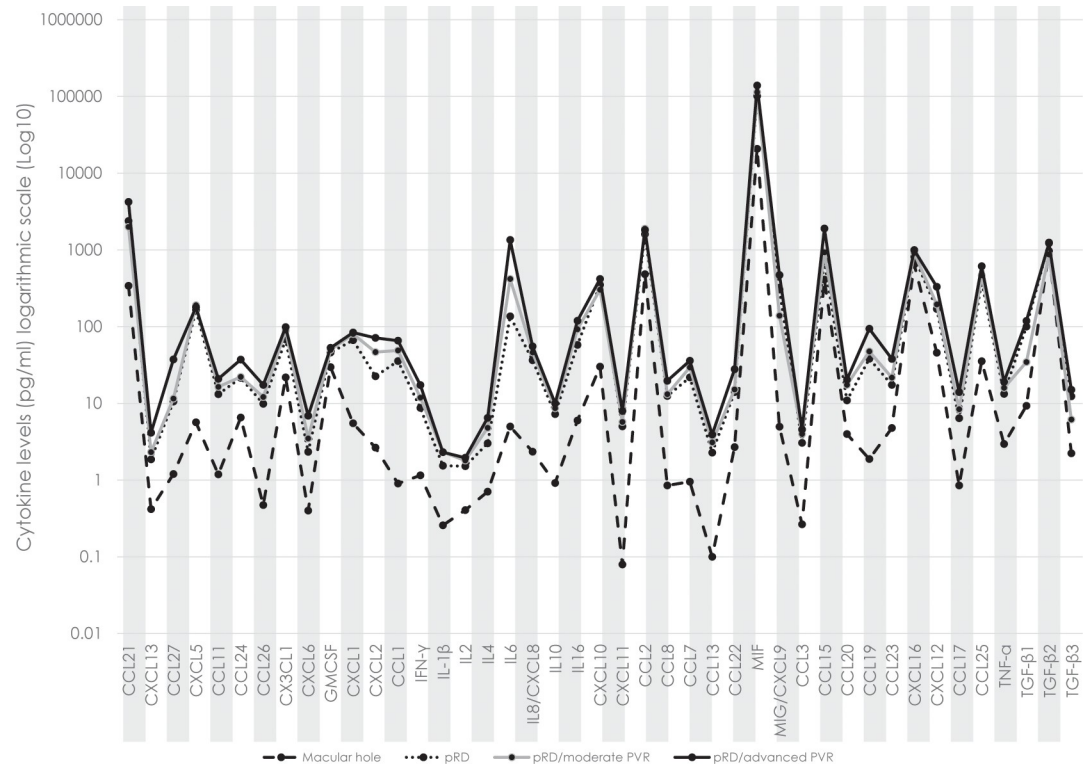


Fig 2. Cytokine levels in the vitreous fluid. Comparing cytokine levels in the vitreous (VF) of eyes of patients with primary retinal detachment without PVR (pRD; $n = 81$) with moderate PVR ($n = 49$), with advanced PVR ($n = 44$), and eyes with macular holes.

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Comparison of cytokine profiles in eyes with pRD without relevant PVR (group 1) and with PVR requiring a gas (group 2) or silicone oil tamponade (group 3)

We found no significant differences in the VF chemo- or cytokine levels between Groups 1 and 2 (Table 1).

In Group 3, however, the concentrations of 13 chemo- and cytokines (CCL27, CCL26, CXCL6, IL-4, IL-16, CXCL10, CCL8, CCL22, MIG/CXCL9, CCL15, CCL19, CCL23, CXCL12) were elevated compared to Group 1 (Table 2). We observed PVR grade of C2 or higher (C2 to D) in 86.4% of patients from Group 3. Comparison of cytokine profiles in eyes requiring gas (group 2) or silicone oil tamponade (group 3) A comparison between Groups 2 and 3 yielded only one cytokine, CCL23, with significantly elevated VF levels in group 3 (Table 3).

Comparing cytokine profiles from eyes with pRD and PVR grade A or B to eyes with PVR grade C1

Patients with pRD with PVR grade B or less displayed one cytokine (CCL19) with elevated levels in comparison to patients with PVR C1, indicating an early change towards more severe PVR. When compared to patients with more severe PVR (grades C2 to D) the latter showed 12 cytokines with significantly higher VF levels: CCL27, CXCL6, IL4, IL16, CXCL10, CCL8, CCL22, MIG/CXCL9, CCL15, CCL19, CCL 23 and CXCL12 (Tables 4–6).

Interestingly, comparing the vitreous levels of eyes with PVR C1 and PVR C2-D, no significant difference in chemo- and cytokine expression was found (Tables 4–6, Fig 3).

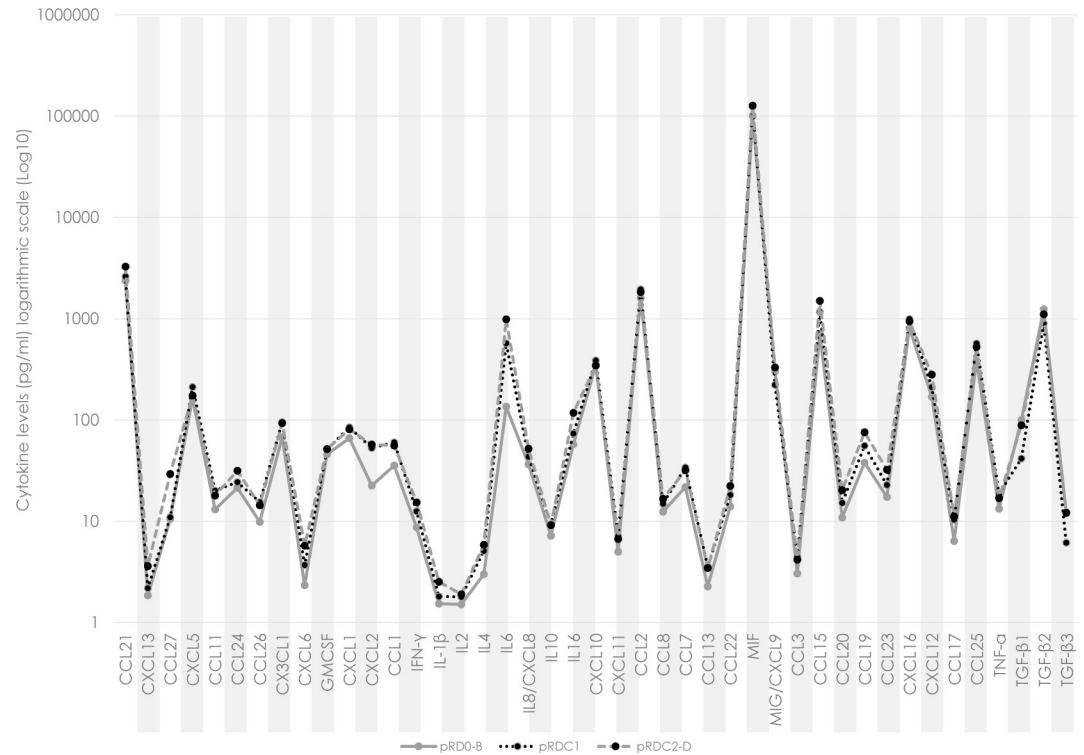


Fig 3. Cytokine levels in the vitreous fluid regarding different PVR grades Cytokine levels in the vitreous (VF) of eyes with primary retinal detachment without PVR or PVR grades A or B (pRD0-B; *n* = 81) with PVR grade C1 (pRDC1; *n* = 27) and grades C2-D (pRDC2-D; *n* = 65).

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Discussion

The comparison of cytokine profiles in different stages of proliferative vitreoretinopathy (PVR) in primary retinal detachment (pRD) identified the chemokine (C-C motif) ligand 19 (CCL19) as specifically upregulated in early PVR (C1). In more advanced stages of PVR (C2-D), 11 additional cytokines (CCL27, CXCL6, IL4, IL16, CXCL10, CCL8, CCL22, MIG/CXCL9, CCL15, CCL 23 and CXCL12) exhibited higher levels in the vitreous fluid compared to vitreous from eyes with pRD, but without PVR. When we compared the cytokine profiles in the pRD group with the most instable retinal situation that received silicone oil (group 3) to that of the group that received gas filling (group 2), CCL23 was the only cytokine elevated in the VF. Since 86.4% of group 3 showed severe PVR (C2-D) this cytokine might qualify as a marker for advanced PVR. This would be supported by the fact that CCL23 is induced by IL-4 and has chemotactic characteristics [25].

We found CCL19 to be the only cytokine upregulated in RD and PVR C1 compared to pRD with PVR grade B or less. CCL19 specifically binds to the chemokine receptor CCR7 which stimulates dendritic cell maturation [26]. Together with CCL21, CCL19 specifically binds to CCR7 and is constitutively expressed to control cell movement during homeostasis. CCR7 and CCL19 are also known to play a role in tissue repair and wound healing [27,28].

Samples of vitreous fluid from eyes with idiopathic epiretinal membranes and/or idiopathic macular holes have served in many studies as controls for cytokine analyses in various ocular pathologies [7,29,30]. Due to fibroblast activity in epiretinal membranes and an elevated inflammatory cytokine profile in the vitreous, we decided to use only patients with macular hole pathology without concomitant epiretinal membranes as a control group [31,32].

When compared to macular holes, the concentrations of almost all tested chemo- and cytokines were elevated in the vitreous of all pRD groups, irrespective of the presence or severity of PVR. This suggests that comparisons using only a single or very few cytokines may be misleading. TGF- β 1- and 2 were the sole cytokines that were not significantly increased. Such unspecific upregulation of many cytokines may result from a damage to the retinovascular barrier following retinal detachment. Therefore, the cytokine milieu changes may represent a timely response to the tissue trauma which cannot be attributed to one single biological process [16,33,34,35]. The significant upregulation of pro-fibrotic and pro-inflammatory cytokines in eyes with RD compared to MH found in our study is in good agreement with previous studies that have found similar upregulations compared to eyes with either macular hole, epiretinal membrane or retinal vein occlusion, notably for cytokines as IL-6 and IL-8 [1,16,19,20,36,37], MCP1, MIP-1 β and IP10 [36,38], and in RD with PVR for IL-6, IL-8, IL-10, TNF, INF- γ , CCL2, CCL3, CCL4, CCL5, CCL11, CCL17, CCL18, CCL19, CXCL9, CXCL19, G-CSF and FGF [2,12,39].

Our intention is a better understanding of the impact of local cytokine environmental changes in the progression of PVR. With the upregulation of 12 out of 43 chemo- and cytokines our findings do not indicate a very targeted or specific local response. This unspecific change in the intraocular environment during the progression from pRD to severe PVR, namely PVR grades C2-D may well explain why many therapeutic attempts to date remained unsuccessful [40,41].

Having identified CCL19 as a possible marker for the early detection of PVR, plus the subsequent upregulation of 11 additional chemo- and cytokines as PVR severity increases, seems to contradict the commonly held notion of this process being primarily unspecific. This raises the hope that we may at some point be able to specifically target the tissue response that results late in the progression of PVR. Currently, it is conceivable that CCL19 could serve as a diagnostic marker for the risk of PVR progression.

The main strength of this study is its well-designed selection process with sufficiently large numbers in each group. While the data is consistent, precise, and reliable, the storage conditions must be regarded as a weak point of this study. Samples were not immediately frozen due to the fact that our operation room is not close to the lab. This may principally allow a partial degradation of distinct peptides, chemo- and cytokines, so that we cannot exclude a minor difference in the absolute cytokine concentrations between sampling and storing. Nevertheless, this process was the same for all samples from all groups, and therefore cannot explain any of the inter-group differences we observed. Moreover, the cytokine concentrations in the aqueous humour and vitreous fluid reported here and in our previous studies [31,42,43] are well in line with the concentrations in the ocular fluids of published data from independent groups [44,45]. One further limitation of this study is the difference in age and gender between the groups. Both are known to play a role in the immune response [46,47,48,49,50,51]. Though their impact is unlikely to account for the differences found here, we have to assume that differences in age and gender might have added to the results.

In conclusion, we could identify substantial (but not targeted) changes in the cytokine profiles in pRD. This lends further support to the importance of unspecific cell activation processes over the course of the disease. However, the cellular source responsible for inducing the increased cytokine concentrations remains to be identified.

Supporting information

S1 Dataset.

(XLSX)

Author Contributions

Conceptualization: Christoph Tappeiner, Justus G. Garweg.

Data curation: Souska Zandi, Peter G. Traine, Alain Despont, Robert Rieben, Magdalena Skowronska.

Formal analysis: Isabel B. Pfister.

Investigation: Souska Zandi, Isabel B. Pfister, Peter G. Traine.

Methodology: Souska Zandi, Isabel B. Pfister, Peter G. Traine, Christoph Tappeiner, Alain Despont, Robert Rieben, Magdalena Skowronska, Justus G. Garweg.

Project administration: Souska Zandi.

Resources: Souska Zandi.

Supervision: Justus G. Garweg.

Writing – original draft: Souska Zandi.

Writing – review & editing: Souska Zandi, Isabel B. Pfister, Peter G. Traine, Christoph Tappeiner, Alain Despont, Robert Rieben, Magdalena Skowronska, Justus G. Garweg.

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