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Intraocular concentrations of cytokines, chemokines, and growth factors in the different forms of retinal detachment and the effect of the macular position

Ph.D. Thesis

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1 List of abbreviations

CTACK: T-cell attracting chemokine

ELISA: Enzyme-linked immunosorbent assay

ERM: Epiretinal membrane

FGF: Fibroblast growth factor

G-CSF: Granulocyte colony-stimulating factor

GM-CSF: Granulocyte-macrophage colony-stimulating factor

GRO-alpha: Growth-related oncogene alpha

HGF: Hepatocyte growth factor

IFN: Interferon

IL: Interleukin

IL-1ra: Interleukin-1 receptor antagonist

IL-2Ralpha: Interleukin-2 receptor alpha

IP-10: Interferon gamma–induced protein 10

IVTA: Intravitreal injection of triamcinolone acetonide

IQR: Interquartile range

LIF: Leukaemia inhibitory factor

MCP: Monocyte chemotactic protein

M-CSF: Macrophage colony-stimulating factor

MH: Macular hole

MIF: Macrophage migration inhibitory factor

MIG: Monokine induced by interferon gamma

MIP: Macrophage inflammatory protein

Beta-NGF: beta-nerve growth factor

PDGF-BB: Platelet-derived growth factor

PDR: Proliferative diabetic retinopathy

PVR: Proliferative vitreoretinopathy

RANTES: Regulated upon activation, normal T cell expressed and secreted

RD: Retinal detachment

RPE: Retinal pigment epithelium

RRD: Rhegmatogenous retinal detachment

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SCF: Stem cell factor

SCGF-beta: Stem cell growth factor beta

SDF-1alpha: Stromal cell-derived factor 1alpha

SD-OCT: Spectral-domain optical coherence tomography

TNF: Tumour necrosis factor

TRAIL: Tumour necrosis factor-related apoptosis-inducing ligand

VEGF: Vascular endothelial growth factor

2 Introduction

Retinal detachment (RD) is the separation of the neurosensory retina from the underlying retinal pigment epithelium (RPE). RD can cause vision loss if untreated, and even with proper surgical intervention, a potentially sight-threatening condition may develop in some cases.

The most difficult challenges for vitreoretinal surgeons are proliferative vitreoretinopathy (PVR) developed from rhegmatogenous RD (RRD) and proliferative diabetic retinopathy (PDR) complicated with tractional RD.

PDR is characterized by neovascularization on the retina and the formation of fibrovascular membranes at the vitreoretinal interface. Complex pathophysiological mechanisms triggered by hyperglycaemia underlie the development of PDR. These mechanisms include hypoxia, the release of inflammatory factors, and vascular endothelial growth factor (VEGF). The development of fibrovascular tissue often leads to hemorrhage and tractional RD (**Figure 1.**). (1)

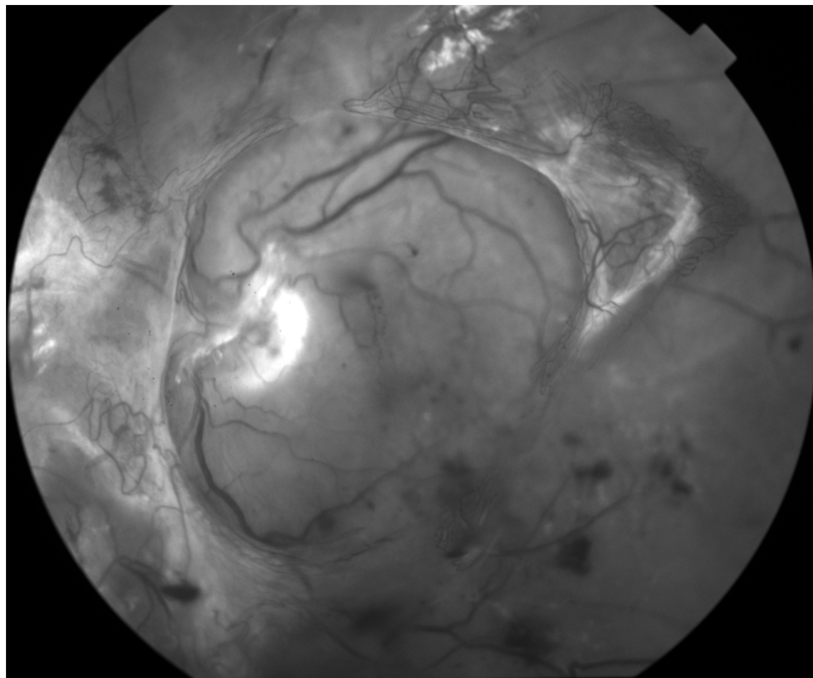

Figure 1. Red-free fundus photograph of tractional retinal detachment. (own photo)

In RRD, liquified vitreous enters under the neurosensory retina through a retinal break (**Figure 2.**). When the vitreous reaches the retinal cells, the affected cells start to secrete factors involved in the destruction and survival of retinal structures. (2)

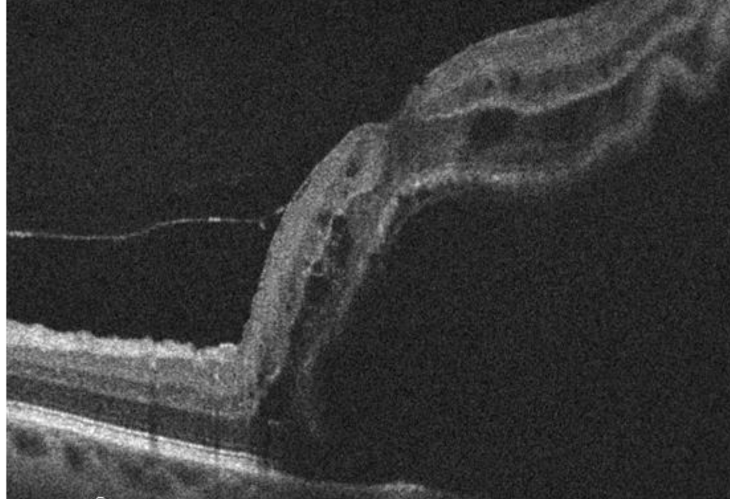

Figure 2. SD-OCT image shows a macula off RRD. (own photo)

Kaufman et al. were among the first to report that macular involvement and duration of RRD were major parameters for postoperative visual acuity. (3) Despite anatomically successful RD surgery resulting in reattached retina visual acuity remains impaired in almost 40% of cases, especially when the macula was detached or PVR developed after surgery. (4) PVR is based on the development of fibrocellular membranes on the surface of and under the retina after RRD, and it occurs in an estimated 5-10 %. (5, 6) Various preoperative and postoperative risk factors for the development of PVR are known. Preoperative risk factors include the existence of large retinal tears, a longstanding retinal detachment, vitreous hemorrhage, aphakia, and choroidal detachment. The intraoperative risk factors that mainly influence the development of PVR include the preoperative existence of PVR, inflammation, vitreous hemorrhage, excessive photocoagulation or cryotherapy, incomplete vitrectomy, undetected breaks. (7) **Figure 3.** shows the main phases of the pathophysiology of PVR, and **Figure 4.** shows a starfold in an eye with PVR.

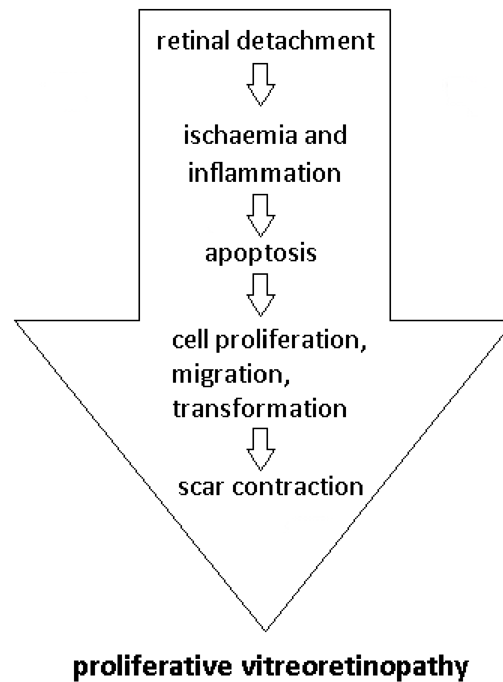

Figure 3. The main phases of PVR. (own figure)

The term PVR was created in 1983 by the Retina Society Terminology Committee which revised the classification Machemer proposed in 1978. (8, 9) In 1991 an updated classification of RD with PVR was also made by Machemer, which is present in Practical Atlas of Retinal Disease and Therapy (**Table 1**.). Machemer was the inventor of the vitreous infusion suction cutter which surgical device made possible the first pars plana vitrectomy (PPV) on 20 April 1970. (10) This surgical approach revolutionized the treatment of RRD and other posterior segment diseases. Moreover, Machemer among others studied the pathophysiology of PVR in animal models.

Table 1. PVR classification by grade. (8)

GRADE	FEATURES
A	Vitreous haze; vitreous pigment clumps; pigment clusters on inferior retina
B	Wrinkling of inner retinal surface; retinal stiffness; vessel tortuosity; rolled and irregular edge of retinal break; decreased mobility of vitreous
C P 1-12	Posterior to equator: focal, diffuse or circumferential full-thickness folds*, subretinal strands*
C A 1-12	Anterior to equator: focal, diffuse or circumferential full-thickness folds*, subretinal strands*, anterior displacement *, condensed vitreous with strands

* Expressed in the number of clock hours involved.

TYPE	LOCATION (IN RELATION TO EQUATOR)	FEATURES
1. Focal	Posterior	Starfold posterior to vitreous base
2. Diffuse	Posterior	Confluent starfolds posterior to vitreous base. Optic disk may not be visible
3. Subretinal	Posterior/ anterior	Proliferations under the retina; Annular strand near disk; linear strands; motheaten-appearing sheets
4. Circumferential	Anterior	Contraction along posterior edge of vitreous base with central displacement of the retina; peripheral retina stretched; posterior retina in radial folds
5. Anterior displacement	Anterior	Vitreous base pulled anteriorly by proliferative tissue; peripheral retinal trough; ciliary processes may be stretched, may be covered by membrane; iris may be retracted

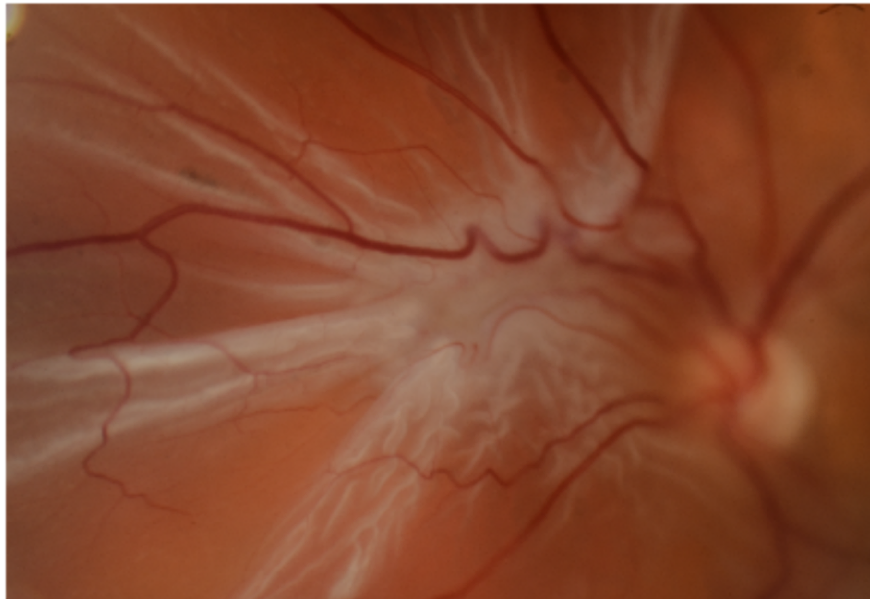

Figure 4. Color fundus photograph of a starfold. (own photo)

Because of the difficulties in the treatment of PVR and PDR, the pathophysiology of these diseases is under extensive research including cytokines, chemokines, and other inflammatory factors. Many studies have reported an immunological component

responsible for PVR, and the formation of tractional RD in PDR. In the first studies, only a few proteins could be assayed in one sample by enzyme-linked immunosorbent assay (ELISA). (11-13) Caepaens et al. were among the first to evaluate three chemokines with ELISA in vitreous samples and found that the MCP-1 level was significantly higher in PVR and PDR compared to controls. (14)

Nowadays a new technique, multiplex bead-based immunoassay provides an opportunity to perform a wide range of molecular analyses in one sample. This helps us understand the interaction between the components of the immunological processes responsible for pathological changes in PDR and PVR. (15, 16) Clinical evidence comparing intraocular cytokine, chemokine, and growth factor levels in patients with PVR, PDR, and RRD is scarce. The role of immunological factors in the pathophysiology of different RDs is important to know to be able to invent new therapeutic targets.

3 Objectives

Our purposes were:

1. Investigation of the intraocular concentrations of cytokines, chemokines, and growth factors in RRD, PVR, and PDR.
   1.1. Exploration of the immunological components of the vitreous that are responsible for the proliferative alterations in PVR and PDR.
   1.2. Gaining more detailed information and compare the differences in the levels of cytokines, chemokines, and growth factors in the vitreous among the different forms of RD.
2. Subgroup analysis and comparison of the intraocular concentrations of cytokines in eyes with PVR, macula on, and macula off RRD.
   2.1. Defining the intravitreal cytokine, chemokine, growth factor patterns of RRD and PVR.
   2.2. Finding correlation of intravitreal cytokine expression with the position of macula lutea and presence of PVR.

Hypotheses:

1. Patients with macula off RRD and PVR have higher levels of cytokines compared to patients with macula on RRD.

2. There is a correlation between intravitreal cytokine expression and the position of the macula and the presence of PVR.

3. An important role in the development of PVR can be attributed to the chemokines involved in the late phase of wound healing.

4 Results

4.1 Intraocular concentrations of cytokines, chemokines, and growth factors in RRD, PVR, and PDR

4.1.1 The immunological components of the vitreous that are responsible for the proliferative alterations in PVR and PDR

Seventy-three eyes of 73 patients undergoing pars plana vitrectomy were included in our cross-sectional study. Patients were divided into four groups according to the indicating ocular pathology: 30 patients with RRD (without PVR), 16 patients with PVR, 8 patients with PDR, and 19 control patients with idiopathic epiretinal membrane (ERM).

Demographic and clinical data are summarized in **Table 2.**

Table 2. Demographic and clinical data of patients. Age, symptom duration, and extent of RD are given in mean ± standard deviation.

		RRD	PVR	PDR	ERM
N (male/female)		30 (18/12)	16 (8/8)	8 (5/3)	19 (5/14)
Age (years)		61 (7.5)	58.4 (11.9)	55 (9.7)	70.7 (8.9)
Symptom duration (days)		7.0 ± 6.4	30.2 ± 28.3	43.4 ± 15.0	NA
Macula on/off		13/17	3/13	2/6	NA
Extent of RD (quadrants)		1.9 ± 0.7	2.9 ± 0.9	2.8 ± 0.8	NA
Location of tears (%)	**Superior**	50	31.2	NA	NA
	Inferior	6.6	56.3	NA	NA
	Temporal	36.7	0	NA	NA
	Nasal	6.6	12.5	NA	NA
Endotamponade (%)	**SF6 gas**	10	18.7	12.5	47.3
	C3F8 gas	73.3	50	50	52.7
	Silicone oil	16.7	31.3	37.5	0

An assay could be performed on all samples. A Kruskal-Wallis test selected 18 out of 48 cytokines, which reached the level of significance in concentration (**Table 3.**). **Table 4.** lists P values, median, and interquartile range (IQR) of concentrations of all individual cytokines in the four patient groups. The most important dependent variables are highlighted below in **Figures 5-8.**

Table 3. Cytokines with significant difference in case of RD. * p<0.05; ** p<0.01; *** p<0.001

	RRD>ERM	PVR>ERM	PDR>ERM	PVR>RRD	PDR>RRD	PDR>PVR
IL-6	***	***	***			
IL-16	**	***	***			
IFN-gamma	***	***	*			
MCP-1	***	***	**			
MIF	***	***	***			
IL-8		**	***		**	
eotaxin		*	***		**	
CTACK			**	*	***	
IP-10		***	***		*	
SCGF-beta				*		
SDF-1alpha		***	***	**	**	
VEGF			*	***		**
IL-18			**		*	
IL-2Ralpha					*	
IL-17					*	
HGF					*	
Beta-NGF					*	*
MIG					**	

Table 4. Median concentrations (pg/ml) and interquartile range of cytokines, chemokines, and growth factors in the vitreous of eyes with PVR, RRD, PDR, and ERM. *: according to Dunn's post hoc test there was no significant difference between the groups

	PVR Median (IQR) pg/mL	RRD Median (IQR) pg/mL	PDR Median (IQR) pg/mL	ERM Median (IQR) pg/mL	P Value
IL-6	63.49 (24.5-181.8)	34.58 (16.98-149.6)	78.36 (39.65-241.5)	9.77 (6.05-13.75)	<0.0001
IL-16	50.1 (26.74-94.51)	32.52 (20.87-61.92)	114.4 (58.91-155.2)	17.13 (12.87-22.68)	<0.0001
IFN-gamma	66.14 (44.42-118.2)	65.21 (42.71-98.81)	59.94 (32.44-154.7)	29.59 (23.66-33.1)	<0.0001
MCP-1	1865 (1182-2499)	1361 (936.7-2209)	1005 (832.2-4365)	399.9 (313.6-543.3)	<0.0001
MIF	3876 (2303-4829)	2550 (1851-3464)	4156 (3052-4971)	780.3 (668.9-1411)	<0.0001
IL-8	83.66 (41.43-173.8)	54.07 (31.75-93.64)	232.2 (123.9-933.9)	29.03 (17.92-48.64)	<0.0001
eotaxin	7.305 (4.598-9.372)	5.2 (3.690-7.673)	10.6 (8.497-15.86)	4.42 (3.2-5.56)	<0.0001
CTACK	69.32 (45.60-98.28)	44.84 (26.15-59.24)	106.9 (77.06-211.5)	47.89 (38.68-62.99)	<0.0001
IP-10	866.6 (575.4-2016)	433.4 (304.5-736.3)	1827 (865.3-3547)	247.4 (154.6-425.3)	<0.0001
SCGF-beta	28963 (14099-56044)	11553 (4115-20960)	21296 (5900-70849)	11256 (7142-19397)	0.0192
SDF-1alpha	209.6 (104.5-272.1)	81.34 (45.68-107)	214.5 (130.7-393)	70.11 (42.57-79.11)	<0.0001
VEGF	225 (208.5-309)	244.7 (170.3-288.7)	614.4 (382.2-893.8)	272.1 (210.7-354.6)	0.0007
IL-18	8.555 (5.65-13.5)	6.65 (5.088-11.13)	18.34 (8.275-25.36)	6.99 (4.31-7.88)	0.0088
IL-2Ralpha	26.41 (12.14-46.32)	15.71 (9.16-24.04)	43.65 (20.47-56.13)	19.87 (14.96-27.01)	0.0087
IL-17	18.61 (10.47-24.43)	15.29 (9.813-23,77)	38.95 (17.11-52.98)	22.93 (10.97-30.93)	0.0518
HGF	7137 (4490-10058)	6208 (4347-9503)	21941 (7807-41857)	10896 (6144-13140)	0.0038
Beta-NGF	11.01 (6.548-13.61)	10.79 (6.95-15.75)	20.48 (13.39-33.47)	18.88 (9-21.66)	0.0123
MIG	186.7 (116.9-296.5)	80.51 (51.09-129,8)	381.6 (179.2-463.7)	247.4 (154.6-425.3)	<0.0001
Basic FGF	426.8 (294.4-566.3)	349.7 (184.2-495.6)	598.9 (329.3-769.4)	475.1 (250-588.2)	0.125
G-CSF	123.3 (70.71-178.5)	100.1 (65.94-138.1)	126.6 (77.13-315.4)	90.79 (63.32-125)	0.2908
GM-CSF	3.62 (2.37-5.79)	4.64 (2.555-5.79)	5.87 (3.45-13.63)	5.79 (3.625-9.5)	0.1383
GRO-alpha	163.7 (134.4-207.9)	168 (134.4-234.9)	152 (129.6-317.7)	152 (132-231.9)	0.9679
IFN-alpha2	22.78 (19.01-32.31)	24.55 (22.78-28.8)	50.67 (29.52-59.2)	29.62 (19.05-40.69)	0.1041
IL-1alpha	23.79 (13.83-34.23)	16.89 (11.44-27.26)	16.9 (7.42-60.51)	25.17 (12.8-39.15)	0.5728
IL-1beta	3.5 (2.03-4.76)	3.64 (2.255-4.69)	3.78 (2.848-9.965)	4.34 (2.988-5.798)	0.4838
IL-1ra	87.21 (53.49-109)	66.27 (43.65-82.41)	89.42 (49.81-183.7)	66.9 (35.16-82.41)	0.1716
IL-2	9.735 (5.893-12.29)	6.745 (3.98-13.36)	9.945 (6.215-23.7)	10.59 (6.105-14.42)	0.4363
IL-3	0.985 (0.56-1.32)	0.91 (0.56-1.445)	1.115 (0.81-2.788)	1.085 (0.635-1.52)	0.4481
IL-4	1.6 (1.13-1.88)	1.6 (1.13-2.06)	2.06 (1.268-3.24)	1.97 (1.6-2.41)	0.2255
IL-5	66.79 (40.96-86.52)	48.37 (30.22-67.43)	69.94 (45.12-117.4)	49.65 (27.59-76.23)	0.1258
IL-7	43.81 (28.55-66.63)	44.41 (25.99-63.64)	44.31 (25.23-73.36)	59.22 (30.46-73.36)	0.6738
IL-9	17.37 (13.09-26.76)	13.99 (10.37-17.24)	17.63 (14.38-36.7)	14.25 (8.18-20.75)	0.1513
IL-10	10.63 (7.63-16.01)	10.62 (6.94-14.36)	11.09 (4.23-28.21)	11.09 (7.4-18.14)	0.9681
IL-12(p70)	17.66 (10.94-29.07)	16.17 (9.8-25.77)	17.29 (5.59-50.96)	21.36 (14.68-39.25)	0.4238
IL-12(p40)	207.2 (139.5-353.3)	235.3 (124.2-313.1)	417.8 (197.1-708)	379.7 (221.3-479.3)	0.0591
IL-13	2.28 (1.143-2.728)	1.93 (1.07-2.49)	3.165 (1.79-4.61)	1.93 (1.07-2.76)	0.1211
IL-15	142.1 (112.7-222.3)	158.9 (126.7-201.8)	175 (124.6-372.4)	206 (126.7-248.2)	0.4141
LIF	50.71 (17.34-65.25)	53.05 (31.61-76.12)	80.69 (34.03-145)	48.35 (20.5-61.2)	0.2529
MCP-3	4.16 (2.235-5.525)	3.53 (1.55-5.61)	3.88 (1.973-10.01)	4.6 (3.35-6.1)	0.5104
M-CSF	27.35 (15.63-30.89)	18.99 (14.58-26.3)	24.21 (12.8-32.97)	22.54 (18.77-29.64)	0.5252
MIP-1alpha	3.1 (1.82-3.648)	2.31 (1.613-2.99)	3.055 (2.213-7.333)	2.18 (1.65-3.1)	0.1786
MIP-1beta	13.59 (1.465-20.49)	5.76 (4.165-17.9)	14.39 (9.13-19.36)	3.05 (2.03-4.07)	0.2569
PDGF-BB	75.19 (61.51-95.15)	74.17 (61.47-93.22)	138.7 (86.24-170.7)	98.09 (72.03-116)	0.0478*
RANTES	19.96 (17.72-30.15)	20.51 (16.28-23.33)	23.73 (20.23-48.21)	24.26 (18.85-31.39)	0.1757
SCF	71.11 (46.41-104.4)	43.17 (28.69-64.15)	47.48 (29.41-59.68)	48.21 (30.87-55.39)	0.0415*
TNF-alpha	21.79 (18.25-30.57)	27.08 (16.46-34.93)	32.32 (20.25-64.52)	28.83 (18.25-42.71)	0.4276
TNF-beta	10.29 (4.9-17.12)	11.45 (5.52-13.18)	14.28 (7.588-34.87)	8.52 (5.96-15.59)	0.7396
TRAIL	13.45 (7.32-16.06)	13.19 (9.208-14.24)	18.38 (13.19-35.99)	10.01 (6.505-15.54)	0.098

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4.1.2 Differences in the levels of cytokines, chemokines, and growth factors in the vitreous among the different forms of RD

Seven cytokines had significantly higher concentrations in the case of all RD groups (RRD, PVR, and PDR) compared to controls: Levels of IL-6 ($p<0.001$, $p<0.001$ and $p<0.001$ respectively), IL-16 ($p<0.01$, $p<0.001$ and $p<0.001$ respectively), IFN-gamma ($p<0.001$, $p<0.001$ and $p<0.05$ respectively), MCP-1 ($p<0.001$, $p<0.001$ and $p<0.01$ respectively), MIF ($p<0.001$, $p<0.001$ and $p<0.001$ respectively) were significantly higher in all groups of RD compared to the group of ERM. The concentrations of IL-8 ($p<0.01$, $p<0.001$, and $p<0.01$ respectively) and eotaxin ($p<0.05$, $p<0.001$, and $p<0.01$ respectively) were significantly higher in PVR and PDR compared to ERM, and significantly lower in RRD compared to PDR. **(Figure 5.).** Further comparisons between groups are summarized in **Table 3**.

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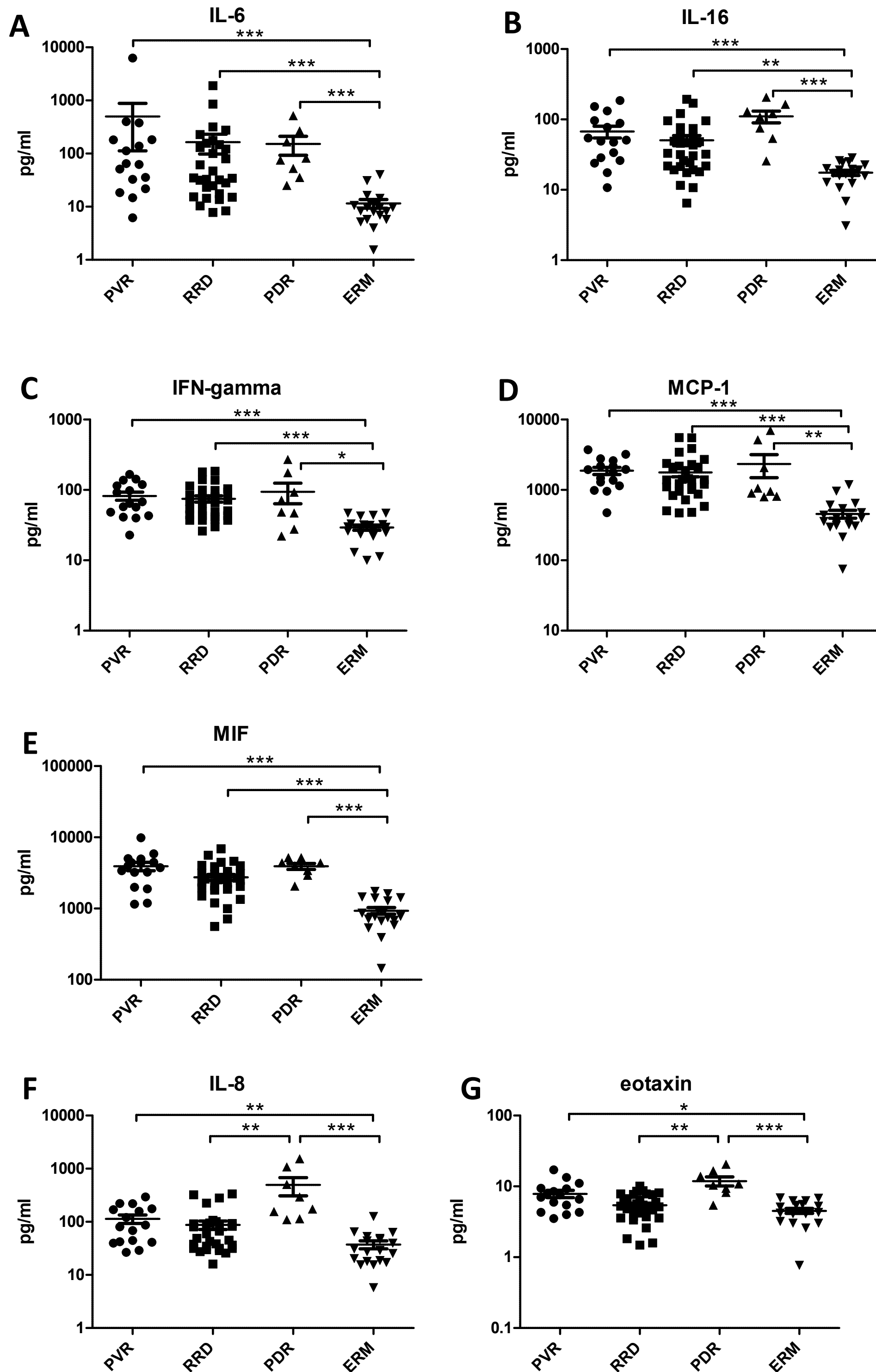

Figure 5. Molecules that had higher concentrations in PVR, RRD, and PDR compared to ERM. Concentrations of IL-6, -16, IFN-gamma, MCP-1, MIF, IL-8, and eotaxin in eyes with PVR, RRD, PDR, and ERM. Statistically significant differences between the groups are marked by asterisks, mean and error bars are indicated. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

There were four cytokines in PDR and PVR groups that had significantly higher levels compared to RRD and ERM **(Figure 6.)**: the level of CTACK was highly increased in patients with PVR (p<0.05 PVR vs RRD) and PDR (p<0.01 PDR vs ERM; p<0.001 PDR vs RRD). Levels of IP-10 were augmented in PDR and PVR vs ERM (p<0.001 both), increased in PDR vs RRD (p<0.05), but not different in PVR vs RRD. SCGF-beta exhibited the highest expression levels in PVR (p<0.05 PVR vs RRD), while not different in PDR vs ERM and RRD. SDF1-alpha was prominent in the PVR (p<0.001 PVR vs ERM; p<0.01 PVR vs RRD) and the PDR (p<0.001 PDR vs ERM; p<0.01 PDR vs RRD) groups.

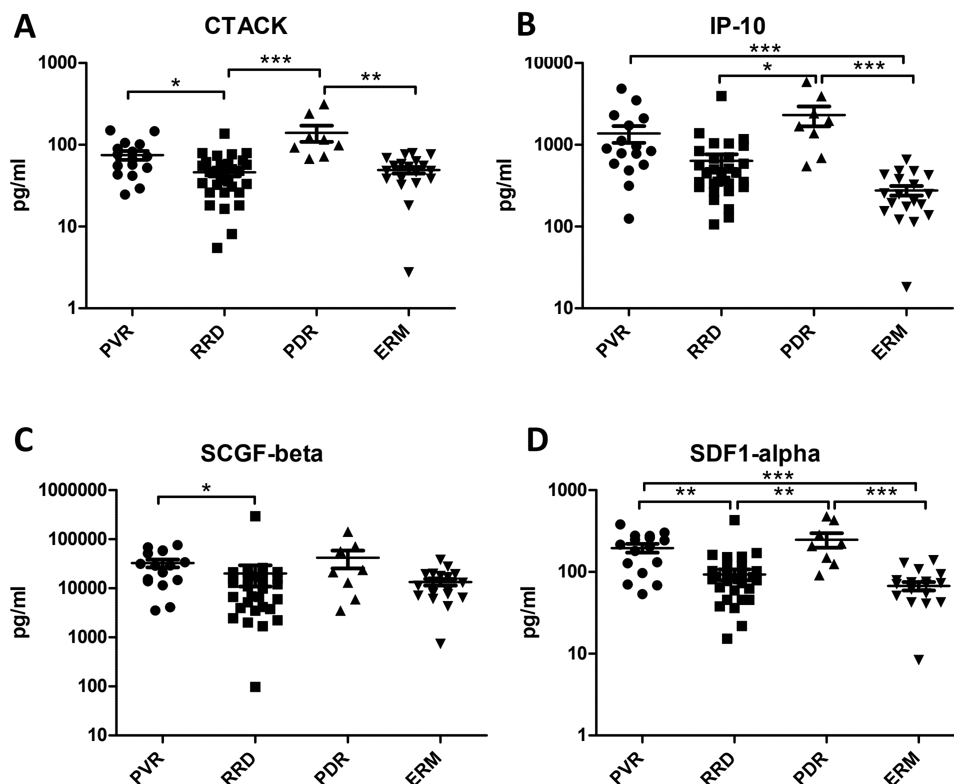

Figure 6. **Molecules with elevated concentrations in PVR and PDR compared to RRD and ERM.**

Concentrations of CTACK, IP-10, SCGF-beta, and SDF1-alpha in eyes with PVR, RRD, RPD, and ERM. Statistically significant differences between the groups are marked by asterisks, mean and error bars are indicated. * p<0.05; ** p<0.01; *** p<0.001

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The concentration values of VEGF in the vitreous fluid were significantly higher in the PDR group (p<0.05 PDR vs ERM; p<0.001 PDR vs RRD and p<0.01 PDR vs PVR). The vitreous level of IL-18 was found to be elevated in the PDR group compared to ERM (p<0.01) and RRD (p<0.05). **(Figure 7**.)

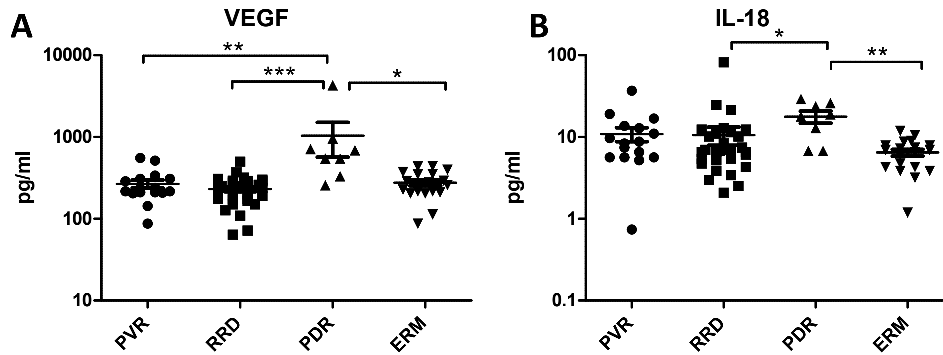

Figure 7. **Molecules with elevated concentrations in PDR compared to PVR, RRD, and ERM.**

Concentrations of VEGF and IL-18 in eyes with PVR, RRD, RPD, and ERM. Statistically significant differences between the groups are marked by asterisks, mean and error bars are indicated. * p<0.05; ** p<0.01; *** p<0.001

Levels of IL-2Ralpha (p<0.05), IL-17 (p<0.05), and HGF (p<0.05) were significantly higher in PDR compared to RRD. The concentration of Beta-NGF was significantly elevated in PDR compared to RRD (p<0.05) and PVR (p<0.05). The levels of MIG were significantly higher in PDR (p<0.01) and ERM (p<0.001) compared to RRD **(Figure 8.).**

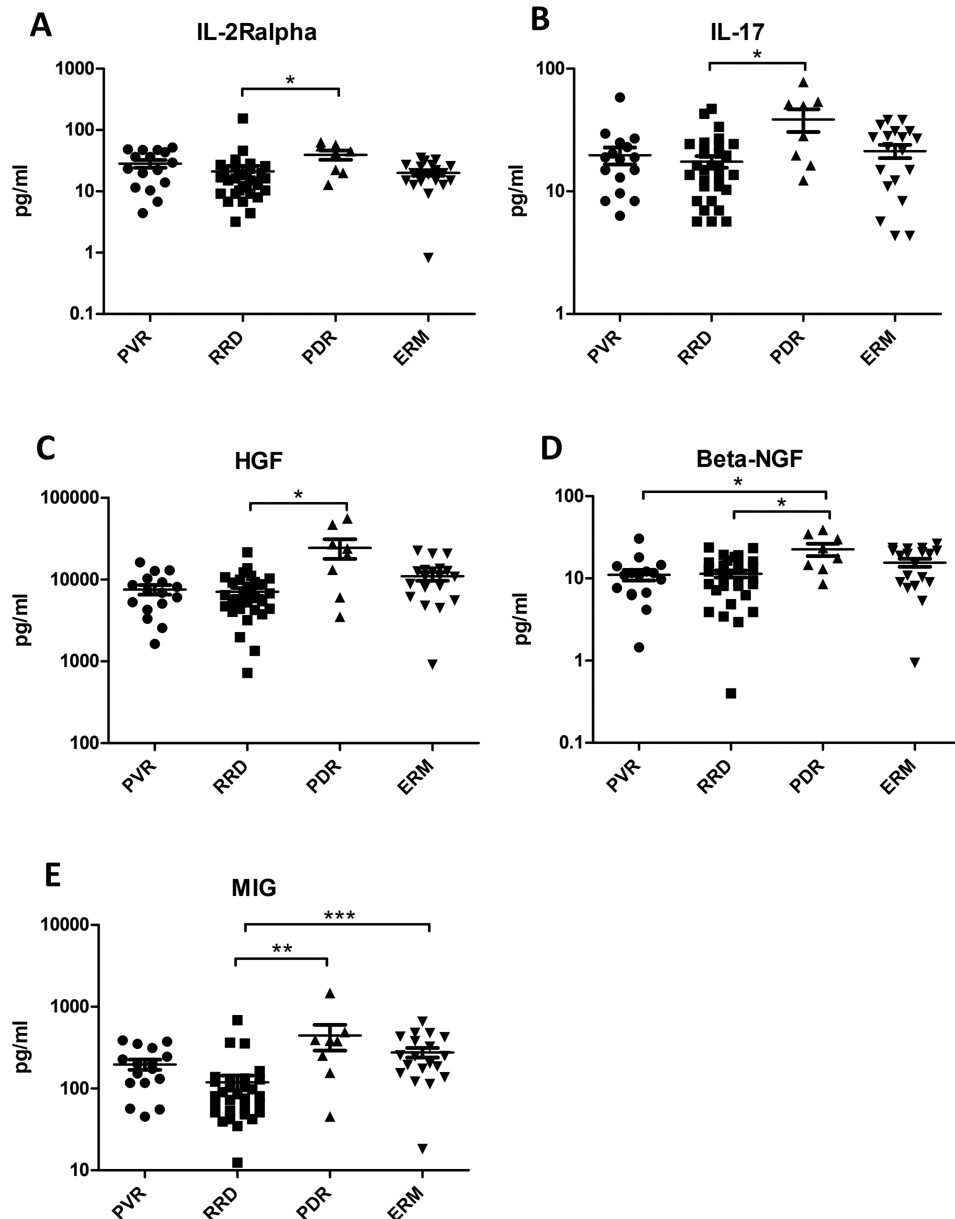

Figure 8. Molecules with elevated concentrations in PDR compared to RRD.
Concentrations of IL-2Ralpha, IL-17, HGF, Beta-NGF, MIG in eyes with PVR, RRD, RPD, and ERM. Statistically significant differences between the groups are marked by asterisks, mean and error bars are indicated. * p<0.05; ** p<0.01; *** p<0.001

4.2 Subgroup analysis and comparison of the intraocular concentrations of cytokines in eyes with PVR, macula on and macula off RRD

Fifty-eight eyes of 58 patients were included in this subgroup analysis. Four groups of patients were formed as follows: a control group consisting of patients without RRD who underwent vitrectomy for the management of ERM, patients with macula off RRD with PVR-C (17), patients with macula off, and patients with macula on RRD without PVR. **Table 5.** shows the patient's demographic data in the groups. The differences in age between the groups were not statistically significant.

Table 5. Demographic data of the patients in the groups.

	PVR	RRD off	RRD on	ERM
N	13	16	13	16
Male/Female	6/7	11/5	8/5	5/11
Age (year)	58.3± 16.3	63.9 ± 7.1	58.6 ± 10.3	68.6 ± 11.6

4.2.1 Intravitreal cytokine, chemokine, growth factor patterns of RRD and PVR

A total of 48 cytokines, chemokines, and growth factors were analysed in the vitreous samples and compared between the four groups. An assay could be performed on all samples; **Table 6.** lists the P values, median, and IQR of concentrations of all individual cytokines in the four patient groups. A Kruskal-Wallis test and Dunn's multiple comparison test selected 24 out of 48 cytokines, which reached the level of significance in concentration.

Table 6. Median concentrations (pg/ml) and interquartile range of cytokines, chemokines, and growth factors in the vitreous of eyes with PVR, macula off and on RRD, and ERM. *: according to Dunn's post hoc test there was no significant difference between the groups

	PVR Median (IQR) pg/mL	RRD off Median (IQR) pg/mL	RRD on Median (IQR) pg/mL	ERM Median (IQR) pg/mL	P Value
HGF	8135 (5695-11547)	7856 (5373-11877)	4730 (3611-7776)	134.4 (124.7-221.8)	<0.0001
IFN-gamma	67.8 (50.06-128.7)	82.14 (54.48-129)	44.23 (37.54-81.51)	29.2 (23.96-32.81)	<0.0001
IL-6	112.3 (43.42-280.8)	40.87 (25.66-208.4)	34.38 (14.03-115.7)	9.77 (6.05-13.75)	<0.0001
IL-8	120.5 (56.42-197.4)	81.52 (39.48-103.7)	34.69 (29.23-80.4)	28.23 (18.12-45.54)	0.0003
IL-16	54.39 (31.24-114.4)	37.46 (22.47-75.03)	48.38 (15.43-75.25)	16.71 (13.29-21.93)	<0.0001
MCP-1	1950 (1218-2687)	1996 (1066-2848)	1107 (798.7-1882)	379.4 (309.5-517.4)	<0.0001
MIF	4371 (3323-4701)	2967 (2036-4030)	2349 (1098-2870)	761.3 (606.9-1201)	<0.0001
CTACK	76.36 (56.23-103.4)	49.04 (27.73-75.62)	34.02 (22.13-51.68)	47.13 (35.19-66.34)	0.0012
Eotaxin	7.91 (6.025-10.25)	6.335 (4.683-8.035)	4.21 (3.465-5.46)	4.42 (3.073-6.1)	0.0006
G-CSF	129.9 (108.4-203.9)	130.7 (94.16-152.7)	76.3 (42.92-106.7)	87.4 (57.18-115.4)	0.0014
IP-10	958.1 (783.5-2208)	529.9 (304.1-1044)	354.8 (303.3-483.7)	249.1 (141.7-408)	<0.0001
MIG	205.3 (142.3-333.1)	104.5 (74.72-137.1)	53.91 (42.38-80.47)	58.07 (38.95-109.2)	<0.0001
SCF	87.46 (51.8-108)	62.55 (40.28-68.26)	31.6 (21.75-44.61)	48.21 (32.32-55.21)	0.0001
SCGF-beta	31569 (18211-58395)	16368 (6636-21651)	6625 (2120-11375)	10018 (6751-18778)	<0.0001
SDF-1alpha	242.9 (138.9-277.2)	94.31 (77.99-159.7)	63.69 (45.68-85.8)	70.11 (42.57-79.11)	0.0002
IL-1ra	97.77 (78.89-111.4)	76.01 (61.91-105.9)	60.72 (30.61-75.25)	73.08 (43.2-84.49)	0.0041
IL-5	73.72 (57.3-101.1)	61.1 (46.12-87.46)	32.84 (26.93-49.01)	47.73 (25.61-70.54)	0.0035
IL-9	22.32 (15.03-28.06)	16.33 (136.1-22.84)	11.92 (7.275-15.5)	14.25 (8.438-19.97)	0.0111
M-CSF	28.8 (17.1-32.35)	23.8 (17.1-32.56)	15 (10.8-22.12)	23.38 (16.89-30.89)	0.0114
MIP-1alpha	3.31 (2.31-3.965)	2.745 (2.018-3.648)	1.65 (1.07-2.43)	2.18 (1.34-2.99)	0.0046
TRAIL	14.76 (11.61-17.09)	13.19 (9.74-16.44)	9.475 (5.123-13.19)	11.6 (7.59-15.54)	0.0209
IL-1alpha	25.17 (16.21-34.94)	23.09 (10.79-32.83)	10.1 (4.775-13.48)	25.17 (11.44-39.15)	0.0202
IL-12(p40)	226 (207.2-370.9)	281.4 (207.2-335.5)	188.2 (101.6-260.5)	423.4 (226-492.1)	0.0174
IL-2Ralpha	36.52 (21.06-47.21)	11.09 (6.488-17.84)	6.49 (3.79-11.09)	19.28 (13.18-27.01)	<0.0001
Basic FGF	451.2 (395.4-598.7)	469 (242.1-574.4)	308.6 (148.6-418.3)	486.8 (253.8-577.2)	0.1397
GM-CSF	4.14 (3.1-6.51)	4.81 (2.37-6.27)	3.01 (2.328-5.205)	4.48 (2.74-7.38)	0.2559
GRO-alpha	163.7 (115.3-207.9)	147.7 (127.1-210.9)	124.7 (96.95-209.5)	134.4 (124.7-221.8)	0.5669
IFN-alpha2	22.78 (19.01-32.31)	23.67 (17.07-33.59)	11.03 (6.125-23.89)	20.94 (17.07-40.33)	0.1208
IL-1beta	4.06 (2.845-5.38)	3.92 (2.775-4.83)	2.03 (1.73-3.78)	4.34 (2.77-5.73)	0.1174
IL-2	10.8 (6.96-13.36)	12.5 (5.255-14.96)	4.83 (3.12-9.73)	10.37 (6.53-15.06)	0.0714
IL-3	1.06 (0.91-1.545)	1.27 (0.635-1.57)	0.71 (0.46-0.86)	1.19 (0.51-1.62)	0.0642
IL-4	1.74 (1.51-1.948)	1.695 (1.245-2.15)	0.83 (0.41-1.6)	1.88 (1.078-2.39)	0.0327*
IL-7	51.35 (36.04-70.14)	49.06 (28.81-69.6)	32.83 (22.03-47.58)	60.33 (32.35-83.88)	0.0556
IL-10	11.55 (8.088-17.43)	11.09 (6.488-16.01)	6.49 (3.79-9.01)	9.93 (5.355-19.57)	0.0536
IL-12(p70)	18.4 (12.07-29.98)	20.99 (7.9-27.24)	12.07 (5.993-15.43)	24.67 (14.87-40.88)	0.0562
IL-13	2.49 (1.93-2.895)	2.35 (1.215-2.963)	1.36 (1.07-2.21)	1.93 (1.07-2.76)	0.1156
IL-15	164.4 (134.5-232.3)	189 (150.6-226.6)	157.1 (126.7-173.2)	197.5 (134.3-245.8)	0.3047
IL-17	18.94 (14.95-25.93)	20.27 (12.47-26.27)	10.97 (6.99-17.28)	22.27 (11.3-33.93)	0.0662
IL-18	9.67 (6.54-15.3)	7.88 (5.7-12.15)	6.54 (4.08-9.68)	6.99 (3.973-7.88)	0.0495*
LIF	55.39 (36.43-70.42)	60.04 (34.03-91.99)	31.61 (7.538-61.18)	55.39 (24.25-64.67)	0.1480
MCP-3	4.6 (3.16-6.26)	3.89 (3.53-6.26)	1.97 (1.438-5.363)	4.6 (1.98-5.94)	0.2215
MIP-1beta	15.18 (6.68-21.16)	11.31 (2.715-20.04)	4.83 (3.845-17.65)	2.03 (0.03-4.07)	0.0704
beta-NGF	11.68 (7.175-14.33)	13.89 (8.09-17.8)	8.54 (3.67-13.01)	19.42 (9-22.78)	0.0504
PDGF-BB	75.19 (61.51-95.15)	74.14 (57.15-111.8)	74.17 (35.3-94.19)	72.03 (35.6-106.2)	0.9538
RANTES	22.13 (19.41-30.9)	23.2 (18-27.11)	18.28 (13-22.13)	24.26 (18.85-32.49)	0.0411*
TNF-alpha	24.44 (20.46-35.79)	27.08 (12.87-36.66)	14.67 (7.41-25.32)	25.32 (16.46-35.8)	0.1338
TNF-beta	10.87 (6.73-20.49)	12.32 (9.405-19.5)	5.52 (4.59-12.31)	7.93 (5.19-13.75)	0.1192
VEGF	239.2 (213.6-323.1)	263.9 (174.9-305.1)	193.1 (138.3-266.7)	265.3 (206.3-335.6)	0.1276

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4.2.2 Correlation of intravitreal cytokine expression with the position of macula lutea and presence of PVR

Levels of six molecules were higher in the case of all RD groups (PVR, macula off, and macula on RRD) compared to the control group. Levels of HGF ($p<0.0001$), IFN-gamma ($p<0.0001$), IL-6 ($p<0.0001$), IL-16 ($p<0.0001$), MIF ($p<0.0001$), MCP-1 ($p<0.0001$) were significantly higher in all groups of RD compared to the group of ERM. The concentration of IL-8 ($p=0.0003$) was significantly higher in PVR and macula off RRD compared to the control group, but we could not find an increase in macula on RRD **(Figure 9.)**. The concentrations of three molecules out of six were higher than 1 ng/ml in all RD groups (median concentrations in PVR: HGF= 8.135 ng/mL, MCP-1= 1.950 ng/mL, MIF= 4.371 ng/mL). **(Table 6.)**

There were eight molecules that had significantly higher levels in PVR compared to macula on RRD and ERM: CTACK ($p=0.0012$), eotaxin ($p=0.0006$), G-CSF ($p=0.0014$), IP-10 ($p<0.0001$), MIG ($p<0.0001$), SCF ($p=0.0001$), SCGF-beta ($p<0.0001$), SDF-1alpha ($p=0.0002$) **(Figure 10.)**. Levels of G-CSF and SCF were additionally significantly higher in macula off RRD compared to macula on RRD **(Figure 10. C, F)**. The concentration of IP-10 was significantly higher in macula off RRD compared to ERM as well **(Figure 10. D)**. SCGF-beta exhibited the highest expression levels in PVR group (median concentration= 31569 pg/mL). Levels of four out of eight molecules were higher than 100 pg/mL (median concentration in PVR: G-CSF= 129.9 pg/mL, IP-10= 958.1 pg/mL, MIG= 205.3 pg/mL, SDF-1alpha= 242.9 pg/mL). **(Table 6.)**

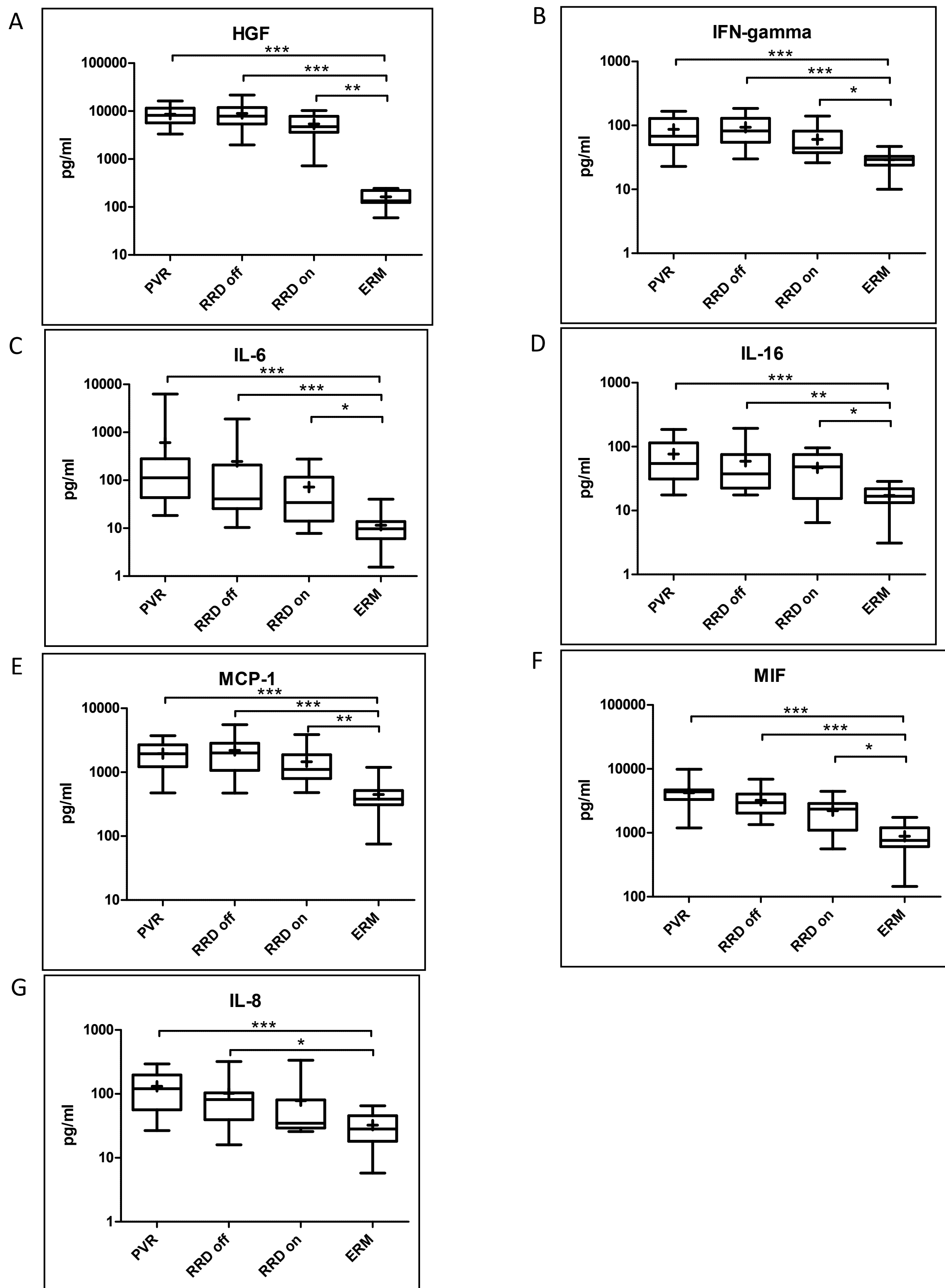

Figure 9. Molecules with elevated concentrations in PVR, macula off, and on RRD compared to ERM. Median and mean (cross) concentrations of HGF, IFN-gamma, IL-6, -16, MCP-1, MIF, and IL-8 in eyes with PVR, macula off RRD, macula on RRD, and ERM. Statistically significant differences between the groups are marked by an asterisk, min-max bars are indicated. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

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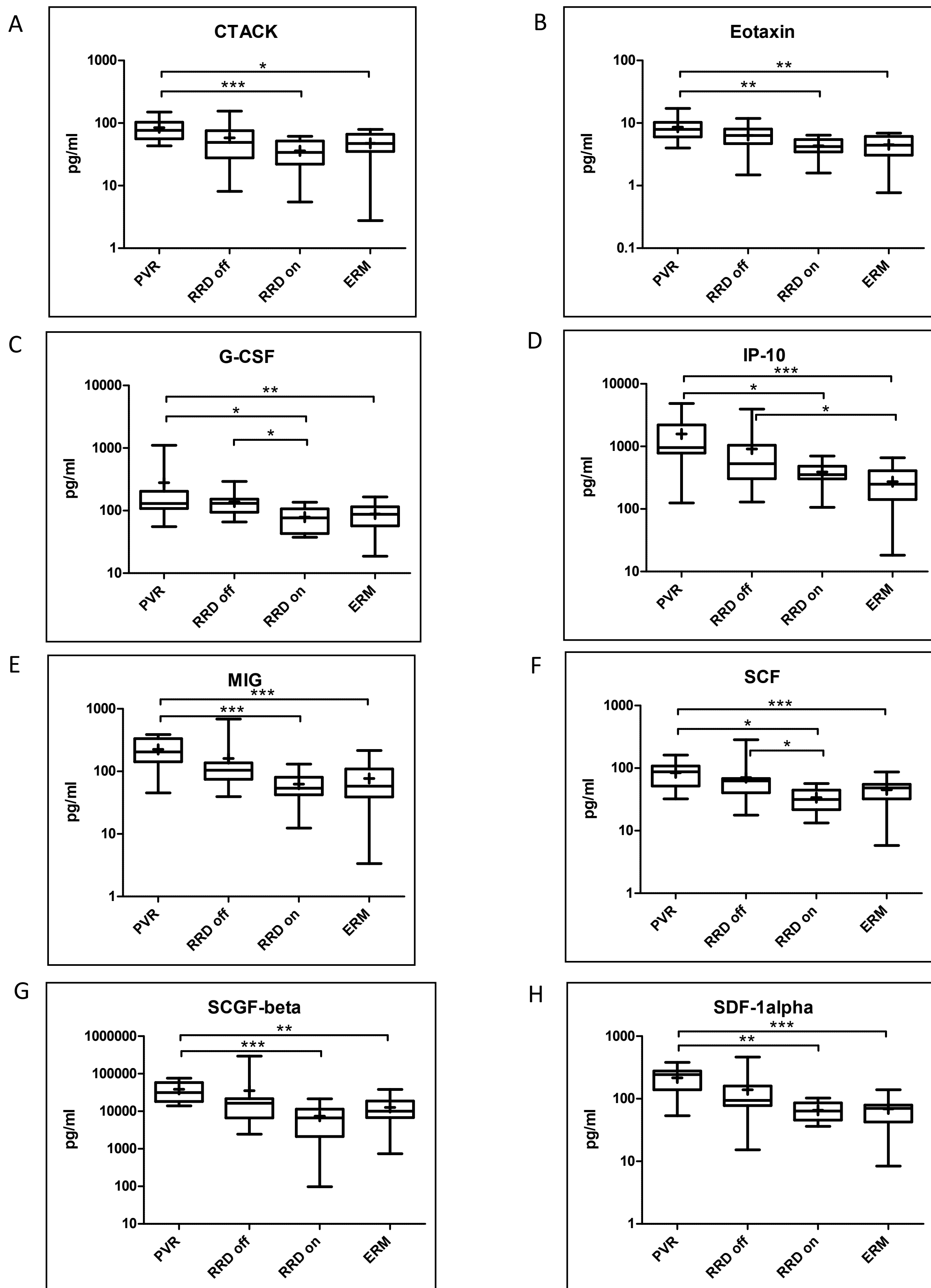

Figure 10. Molecules with elevated concentrations in PVR compared to macula on RRD and ERM. Median and mean (cross) concentrations of CTACK, eotaxin, G-CSF, IP-10, MIG, SCF, SCGF-beta, SDF-1alpha in eyes with PVR, macula off RRD, macula on RRD, and ERM. Statistically significant differences between the groups are marked by an asterisk, min-max bars are indicated. * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Concentration of six molecules were significantly higher in PVR compared to macula on RRD: IL-1ra (p=0.0041), IL-5 (p=0.0035), IL-9 (p=0.0111), M-CSF (p=0.0114), MIP-1alpha (p=0.0046), TRAIL (p=0.0209) **(Figure 11.)**.

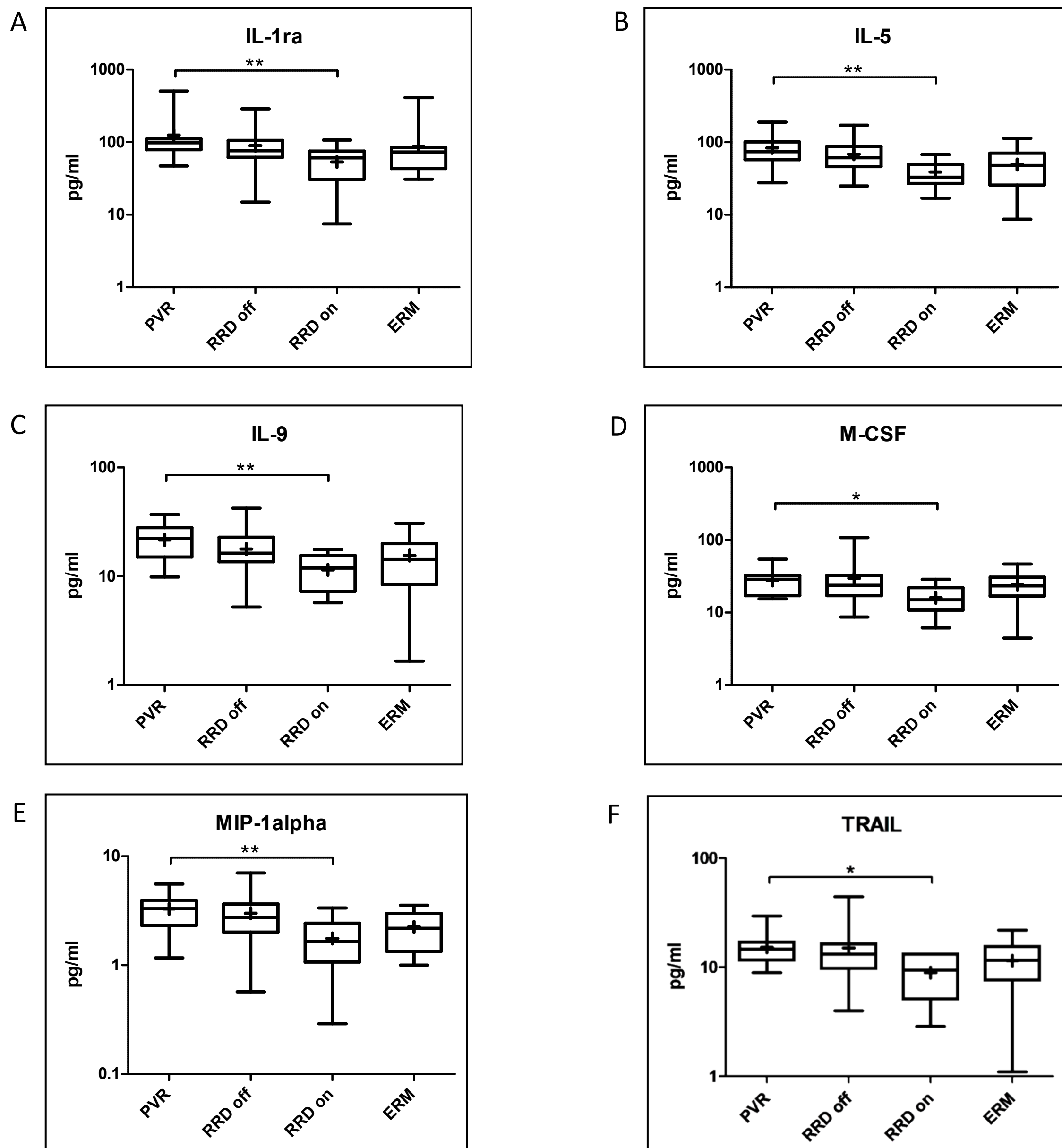

Figure 11. Molecules with elevated concentrations in PVR compared to macula on RRD. Median and mean (cross) concentrations of IL-1ra, -5, -9, M-CSF, MIP-1alpha, TRAIL in eyes with PVR, macula off RRD, macula on RRD, and ERM. Statistically significant differences between the groups are marked by an asterisk, min-max bars are indicated. * $p<0.05$; ** $p<0.01$

We found that the concentrations of three molecules were significantly lower in macula on RRD compared to ERM: IL-1alpha (p=0.0202), IL-12(p40) (p=0.0174), IL2-Ralpha (p<0.05). **(Figure 12., 13.)** The level of IL2-Ralpha was significantly higher in PVR compared to macula off and macula on RRD (p<0.0001) as well **(Figure 13.)**.

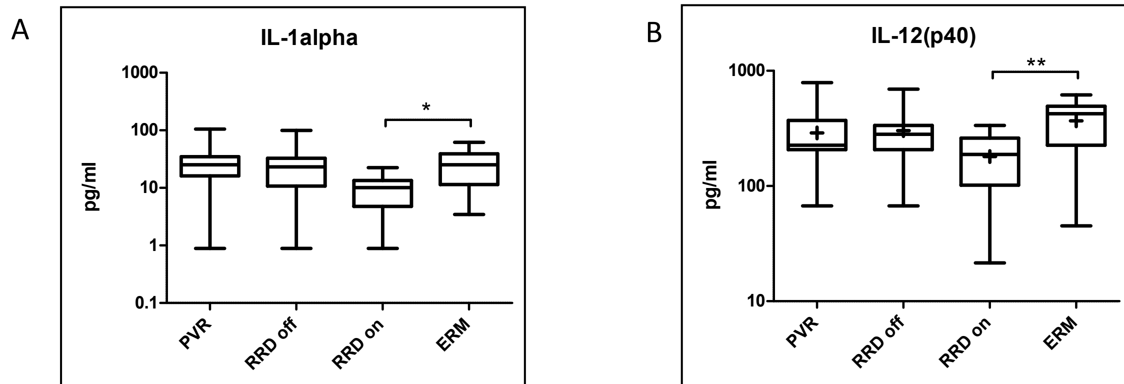

Figure 12. The concentration of cytokines that were significantly lower in macula on RRD compared to ERM.

Median and mean (cross) concentrations of IL-1alpha, IL-12(p40) in eyes with PVR, macula off RRD, macula on RRD, and ERM. Statistically significant differences between the groups are marked by an asterisk, min-max bars are indicated. * p<0.05; ** p<0.01

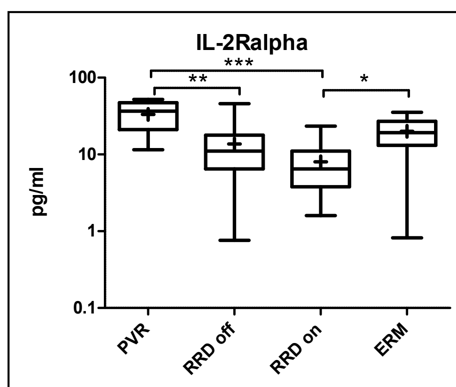

Figure 13. The concentration of IL-2 Ralpha was significantly higher in PVR compared to macula off and on RRD, and it was significantly lower in macula on RRD compared to ERM. Median and mean (cross) concentrations of IL-2Ralpha in eyes with PVR, macula off RRD, macula on RRD, and ERM. Statistically significant differences between the groups are marked by an asterisk, min-max bars are indicated. * p<0.05; ** p<0.01; *** p<0.001

5 Discussion

As a result of the difficulties in the management of RD, many groups are working on exploring the possible non-surgical treatment of PVR. Pennock et al. proposed that ranibizumab might be potential prophylaxis for PVR. They discovered that ranibizumab reduced the bioactivity of vitreous of patients and experimental animals with PVR, and protected rabbits from developing the disease. (18) Other groups studied further agents that may be effective in the treatment of PVR. Kunikata et al. investigated the role of intravitreal injection of triamcinolone acetonide (IVTA) in preventing photoreceptor apoptosis in eyes with RRD. They discovered that IVTA suppressed elevated levels of aqueous humor MCP-1, MIP-1β, and IP-10 in eyes with RRD. (19) Asaria et al. found that adjuvant 5-fluorouracil and low molecular weight heparin significantly reduce the incidence of postoperative PVR. (20) Sadaka et al. evaluated intravitreal methotrexate infusion during pars plana vitrectomy for RRD with a high risk of PVR. They concluded that eyes at high risk for PVR had a low incidence of PVR formation following intravitreal methotrexate infusion. (21) Kawahara et al. suggested that statins could be potent inhibitors of cicatricial contraction in proliferative vitreoretinal diseases. They found that intravitreal injection of simvastatin dose-dependently prevented the progression of diseased states in an in vivo model of PVR. (22) Mysore et al also studied the effect of statins in cultures of human RPE cells before the induction of PVR. They suggest that intravitreal statin therapy may have the potential in alleviating the risk of post-surgical PVR. (23) Some groups established animal models of PVR that allow extensive functional studies and drug testing. Márkus et al. studied the role of transglutaminase 2 in a knockout mouse model of PVR, and they found that the lack of transglutaminase 2 did not prevent the formation of PVR. (24) Heffer et al showed that a single intravitreal injection of the polyether ionophore salinomycin effectively inhibited the formation of PVR in a mouse model. Immunohistochemistry analysis showed that salinomycin treatment reduced both fibrotic and inflammatory markers compared to control treatment. (25) Despite these findings, there is no available cure or prophylaxis for PVR as of yet, apart from the surgical approach. (26) In the treatment of PDR, pars plana vitrectomy plays the main role with various microsurgical techniques.

Iyer et al. proposed a surgical algorithm for the management of PDR with tractional RD based on their compilation of relevant literature. (27)

Although there are a number of surgical adjunctive agents listed above for preventing the development of PVR, all have limited efficacy. It is important to discover predictive molecular biomarkers to determine the probability of PVR development after retinal reattachment surgery. (7)
Our results show that common vitreous biomarkers involved in RDs are IL-6, IL-8, IL-16, IFN-gamma, MCP-1, and MIF. This reveals the strong inflammation component in the pathology of RD. However, different RD types show a phenotype-dependent profile in the expression of cytokines.

The interpretation of our data is challenging due to the complexity of the molecules and RD pathomechanism. Previous studies analysed with similar methods, but different aspects of RD.
Abu El-Asrar et al. measured the levels of ten chemokines with ELISA in the vitreous from eyes undergoing pars plana vitrectomy for the treatment of RRD, PVR, and PDR and they concluded that MCP-1, IP-10, and SDF-1 may be involved in the pathogenesis of PVR and PDR. Our results are consistent with Abu El-Asrar's, but we could analyse a wider range of molecules in each sample with the help of multiplex bead-based immunoassay. (13) Wang et al. showed that levels of IL-6 and MCP-1 were significantly higher in vitreous and aqueous humour in patients with PDR compared to controls with macular holes (MH) (28). Dai et al. documented that MCP-1, MIP-1beta, IP-10, MIG, and VEGF levels were increased in PDR compared to ERM and MH. (29) The same proteins were augmented in the vitreous of our PDR samples. Moreover, CTACK and eotaxin levels were prominent in our vitreous samples. In PDR pathology chemoattraction seems to be active, but as a disease characteristical sign, increased angiogenesis through VEGF can be observed, as previously shown. Note, that elevated VEGF levels were not detected in PVR pathogenesis. The role of VEGF in diabetic macular edema and PDR is well known. (30) We observed that levels of IL-18 and VEGF were significantly higher in PDR. Song et al. documented that the levels of intravitreal VEGF and IL-18 were significantly higher in active PDR compared to ERM and MH. (31) Xu et al. found that the vitreous levels of CCL2, CXCL4, CXCL9,

CXCL10, VEGF, sVEGFR-1, sVEGFR-2, IL-6, IL-8, IL-10, and IL-18 were elevated significantly in the PDR group compared to nondiabetic patients. (32) In our study, we found a significant elevation in the levels of IL-18 in PDR compared to the control group and RRD separately. Increased IL-18 expression levels in vitreous fluid reveal inflammasome activation. (33) Inflammasomes are large cytosolic protein complexes composed of Nod-like receptor sensor protein, adaptor protein ASC and caspase, mainly caspase-1, as an effector enzyme. (34) Inflammasome activation results in the release of pro-inflammatory cytokines of IL-1beta and IL-18. Since, inflammasomes seem to be activated during the late state of the PDR process they might be a good therapeutic target to prevent tractional RD, once current therapy drugs have not helped anymore.

Takahashi et al. characterized the expression profiles of 27 cytokines in the vitreous of patients with RRD compared to PDR, retinal vein occlusion, MH, and ERM. The levels of IL-6, IL-8, MCP-1, IP-10, MIP-1beta were significantly higher in RRD compared to the control group. (35) These results are similar in our study when ERM cases were used as controls. They also found higher IL-6 and IL-8 levels, but not MCP-1 and IP-10, in RRD rather than PDR. This reveals a stronger chemoattraction in PDR with tractional detachment.

In our study, we could not detect a significant increase in the concentration of VEGF in RRD, but Rasier et al. demonstrated increased levels of IL-8 and VEGF in vitreous samples from eyes with RRD compared to MH and ERM. (36) Ricker et al. documented that IL-1alpha, -2, -3, -6, VEGF, and ICAM concentrations are increased in the subretinal fluid of PVR but not in RRD. (37) We show here that the expression of VEGF was significantly higher only in the PDR group compared to RRD, PVR, and ERM. Our results are consistent with the previous reports. (35, 38) It seems that VEGF has the strongest biomarker role in PDR with and without tractional detachment. Interestingly, levels of CTACK, IP-10, and SDF1-alpha were significantly higher in PVR and PDR compared to RRD, while stem cell factor SCGF-beta was more present in PVR rather than in RRD. Keles et al also found high levels of SDF-1alpha, VEGF, and angiopoietin-like protein 2 in eyes with PDR corresponding with our results. (39) CTACK, IP-10, and SDF-1 play a role in a wide variety of processes such as

chemotaxis, immune response, cell-cell signalling, differentiation, and activation of peripheral immune cells, regulation of endothelial cell proliferation.

Additionally, we performed a subgroup analysis in 42 patients with RRD and 16 age-matched controls with ERM to investigate if there is a difference in the cytokine profile of macula off, macula on RRD, and PVR. Our study results demonstrated that the vitreous of eyes with macula on RRD contains a substantially lower concentration of half of the analysed molecules. We are the first to report that there is a difference in the cytokine pattern of the vitreous of patients with macula off and macula on RRD. In macula on RRD, the concentrations of 15 molecules were significantly lower compared to PVR. Significant differences were found between macula on and macula off RRD in the concentrations of G-CSF and SCF.

SCF is a potent synergistic growth factor in haematopoiesis and results in augmentation of the proliferation, differentiation, and survival of haematopoietic cells. (40, 41) SCF synergy with G-CSF has important biological and clinical significance. Duarte et al. investigated the signaling pathways SCF promotes G-CSF. Cell cycle analysis revealed that increased proliferative state induced by SCF and G-CSF cotreatment was associated with the direct effect of these cytokines on cell cycle distribution. (42) The inflammatory character and synergistic effect on other chemokines of these molecules might have an impact on the physiology of retinal cells that contributes to impaired visual acuity in macula off RRD despite anatomically successful surgery.

We found that the concentrations of eight molecules (CTACK, eotaxin, G-CSF, MIG, IP-10, SCF, SCGF-beta, SDF-1alpha) were significantly higher in PVR compared to macula on RRD and ERM. These chemokines have a key role in the recruitment and function of T-lymphocytes, (43) and there are complex connections between them. From these eight chemokines, SCGF-beta reached the highest level from all the measured molecules. SCGF-beta has a burst-promoting activity and a granulocyte-macrophage (GM) colony-promoting activity on erythroid and GM progenitor cells (44) and acts synergistically with other cytokines, including G-CSF, GM-CSF and has a connection with CTACK, SCF, and IL-16 according to the string database. The concentrations of four out of eight molecules were higher than 100 pg/ml: G-CSF, IP-

10, MIG, SDF-1alpha. IP-10 and MIG bind to the same receptor (CXCR3). (45, 46) The CXCR3 chemokine receptor regulates the migration of Th1 lymphocytes and responds to three ligands: MIG (CXCL-9), IP-10 (CXCL-10), and I-TAC (CXCL11). (47)

Chemokines play a role in wound healing. Early wound healing includes hemostasis, inflammation, and proliferation. Late wound healing is the remodelling stage. IP-10 and I-TAC play a role in the proliferation and remodelling stage. IL-8 (CXCL-8) plays a role in inflammation, MCP-1 (CCL-2) participates mainly in the inflammation and proliferative phase of early wound healing. IFN-gamma plays a role in angiogenesis. SDF-1alpha (CXCL-12) is present in all early phases of wound healing, including the proliferation phase. (48) Levels of cytokines that are mainly present in the early phase were increased in all of the RD groups, but the concentration of IP-10 that participates in the proliferative and remodelling phase was elevated only in the macula off RRD and PVR group. Our findings indicate that in the pathophysiology of PVR, those chemokines have a key role that participates in wound healing, especially in the late phase.

The concentrations of HGF, IFN-gamma, IL-6, IL-16, MIF, MCP-1 were significantly higher in all groups of RD compared to controls. The level of IL-8 was significantly higher in macula off RRD and PVR compared to ERM. HGF, MIF, and MCP-1 had higher concentrations than 1 ng/ml in the vitreous of macula on, macula off RRD, and PVR.

HGF is one of the cytokines constitutively produced by human bone marrow (BM) stromal cells and indirectly promotes haematopoiesis. (49) Matsuda-Hashii et al. studied the effect of HGF on stromal cells. They revealed that HGF is an autocrine regulator, which can maintain the hematopoietic microenvironment through stimulating proliferation and adhesion to the extracellular matrix and promoting hematopoiesis through inducing constitutive production of IL-11, SDF-1alpha, and SCF. (50) Lashkari et al. investigated the role of HGF in the formation of PVR in human donor eyes. They concluded that HGF is a potent chemoattractant for cultured human RPE cells, HGF and HGF receptor might play a role in the normal function of RPE cells and RPE-related diseases such as PVR. (51) Briggs et al. searched the presence of HGF in PVR

membranes, in the vitreous and the subretinal fluid of eyes with PVR. They found that RPE cells respond by shape change and cell migration to HGF. (52)

Previous studies have explored molecular alterations in RRD and PVR. Pollreisz et al. explored cytokines and chemokines that were significantly upregulated in the vitreous of RRD eyes compared with ERM, including IL-6, IL-8, MCP-1, IP-10. (2) Josifovska et al. studied 105 inflammatory cytokines in the subretinal fluid of 12 patients with RRD. They found that 37 of the studied cytokines were significantly higher in the subretinal fluid of RRD patients compared to the vitreous of non-RRD patients. (53) Wladis et al. documented ten molecules that were statistically significantly different in PVR compared to primary RRD and ERM. The levels of IP-10, SCGF, SCF, G-CSF were higher in PVR compared to RRD and ERM in parallel with our study. (38) It seems that chemoattraction plays a central role in the pathogenesis of PVR when IL-8 and IP-10 are used as biomarkers. Upregulation of IL-6 and SCGF reveals that our PVR samples represent a late state process with chronic inflammation and fixed retinal folds. Roybal et al. revealed that in late PVR vitreous, cytokines driving mainly monocyte responses and stem-cell recruitment (SDF-1). (54)

Garweg et al. documented that the levels of 39 of 43 cytokines in the vitreous and 23 of 43 cytokines in the aqueous humour were significantly higher in eyes with RRD than in those with MH and they could not find relevant differences in the cytokine profiles of phakic and pseudophakic eyes. (55) Zandi et al. evaluated the same 43 cytokines in RRD, moderate, and advanced PVR compared to MH. They revealed that eyes with PVR C2-D showed higher levels of CCL27 (CTACK), CXCL12 (SDF-1), CXCL10 (IP-10), CXCL9 (MIG), CXCL6, IL-4, IL-16, CCL8 (MCP-2), CCL22, CCL15 (MIP-1delta), CCL19 (MIP-3beta), CCL23 and compared to controls. Interestingly, no difference in cytokine levels was detected between C1 and C2-D PVR. (17) They concluded that CCL19 may represent a potential biomarker for early PVR progression. (56)

Though our study has some limitations, such as the complexity and a high number of cytokines that need further investigations to detect their relationships more exactly. RD and PDR present with variable clinical features, which might contribute to the multiplex

variations of cytokines in the fluids. In addition, it can not be identified whether the concentrations of cytokines are elevated in the vitreous due to the RD (as a consequence) or they are already present before the detachment (as a causative agent). This limitation is hard to solve due to ethical reasons since the human vitreous of healthy eyes is not accessible in everyday routine clinical care.

Given the corresponding results in the levels of cytokines in RRD and PVR in the different studies, they may represent novel therapeutic targets in the management of these diseases. According to our analysis and previous studies HGF, IFN-gamma, IL-6, IL-8, MCP-1, MIF, IP-10 may serve as biomarkers for RRD. CTACK, G-CSF, MIG, IP-10, SCF, SCGF-beta, and SDF-1alpha may participate in the pathogenesis of PVR and represent potential biomarkers for PVR. Higher levels of SCF and G-CSF in macula off RRD compared to macula on RRD may reveal molecular pathways that participate in the poorer prognosis of macula off RRD despite anatomically successful surgery.

6 Conclusions

We conclude, that our results indicate that complex and significant immunological mechanisms are associated with the pathogenesis of different forms of RD such as RRD, PVR, and PDR. Concentrations of cytokines, chemokines, and growth factors are elevated in the vitreous of eyes with RD, the increase is dependent on the form of RD. The detected proteins are present in different concentrations both in RRD and PVR. In the presence of PVR and PDR, levels of the majority of cytokines are significantly elevated, thus they may serve as biomarkers to estimate the progression or severity level of proliferation. Our study adds new biochemical information to the previous studies in correlation with proliferative vitreoretinal alterations. The more exact knowledge of levels of vitreal cytokines may represent novel, therapeutic targets in the management of these diseases. Future investigations should focus on identifying the potential biomarkers to be able to intervene before irreversible proliferative alterations occur.

6.1 Intraocular concentrations of cytokines, chemokines, and growth factors in RRD, PVR, and PDR

6.1.1 Exploration of the immunological components of the vitreous that are responsible for the proliferative alterations in PVR and PDR

To our knowledge, our reports are the first to simultaneously evaluate the concentrations of these 48 cytokines, chemokines, and growth factors in different forms of RD, including RRD, PVR, and PDR with tractional RD.

The concentration of seven cytokines was elevated in RD compared to controls: IL-6, IL-16, IFN-gamma, MCP-1, MIF. The concentrations of IL-8 and eotaxin were significantly higher in PVR and PDR compared to ERM, and significantly lower in RRD compared to PDR. Levels of CTACK, IP-10, SCGF-beta, and SDF-1-alpha were increased in PDR and PVR groups compared to RRD and ERM.

6.1.2 Gaining more detailed information and compare the differences in the levels of cytokines, chemokines, and growth factors in the vitreous among the different forms of RD

The concentration of VEGF and IL-18 were higher in PDR. Levels of IL-2Ralpha and HGF were higher in PDR compared to RRD. The concentration of Beta-NGF was significantly elevated in PDR compared to RRD and PVR. The levels of MIG were higher in PDR and ERM compared to RRD.

6.2 Subgroup analysis and comparison of the intraocular concentrations of cytokines in eyes with PVR, macula on, and macula off RRD

6.2.1 Intravitreal cytokine, chemokine, growth factor patterns of RRD and PVR

Furthermore, we are the first to publish that there is a difference in the cytokine pattern of the vitreous of patients with macula off and macula on RRD. In macula on RRD, the concentrations of 15 molecules were significantly lower compared to PVR. Significant differences were found between macula on and macula off RRD in the concentrations of G-CSF and SCF.

6.2.2 Correlation of intravitreal cytokine expression with the position of macula lutea and presence of PVR

Comparison of the levels of intravitreal cytokines, chemokines, and growth factors of eyes in correlation with the position of the macula lutea (macula on, macula off RRD, and PVR).

Levels of HGF, IFN-gamma, IL-6, IL-16, MIF, and MCP-1 were increased in the case of all RD groups compared to the control group. The concentration of IL-8 was higher in PVR and macula off RRD compared to the control group, but not in macula on RRD.

In PVR compared to macula on RRD and ERM: CTACK, eotaxin, G-CSF, IP-10, MIG, SCF, SCGF-beta, SDF-1alpha were elevated. Levels of G-CSF and SCF were elevated

in macula off RRD compared to macula on RRD. The concentration of IP-10 was significantly higher in macula off RRD compared to ERM as well.

In PVR compared to macula on RRD concentrations of IL-1ra, IL-5, IL-9, M-CSF, MIP-1alpha, and TRAIL were higher.

Concentrations of IL-1alpha, IL-12(p40), and IL2-Ralpha were significantly lower in macula on RRD compared to ERM. The level of IL2-Ralpha was significantly higher in PVR compared to macula off and macula on RRD.

Hypotheses:

Our data supported all our hypotheses.

1. Patients with macula off RRD and PVR have higher levels of cytokines compared to patients with macula on RRD.
2. There is a correlation between intravitreal cytokine expression and the position of the macula and the presence of PVR.

Concentrations of 15 out of 48 cytokines were significantly higher in PVR compared to macula on RRD: CTACK, eotaxin, G-CSF, IP-10, MIG, SCF, SCGF-beta, SDF-1alpha, IL-1ra, IL-5, IL-9, M-CSF, MIP-1alpha, TRAIL, and IL2-Ralpha.

Levels of G-CSF and SCF were significantly higher in macula off RRD compared to macula on RRD as well.

These elevated cytokines in PVR and macula off RRD compared to macula on RRD support the hypothesis that there is a correlation between intravitreal cytokine expression and the position of the macula and the presence of PVR.

3. An important role in the development of PVR can be attributed to the chemokines involved in the late phase of wound healing.

The concentrations of cytokines that are mainly present in the early phase were increased in all of the RD groups, but the level of IP-10 that participates in the proliferative and remodelling phase was higher only in the macula off RRD and PVR group. Our findings indicate that in the pathophysiology of PVR, those chemokines have a key role that participates in wound healing, especially in the late phase.

7 Summary

The purpose of our study was to explore the immunological components that are responsible for the proliferative alterations in the different forms of RD and to compare the concentrations of intravitreal cytokines, chemokines, and growth factors between macula on, macula off RRD, and PVR.

Vitreous fluids were collected during 23G pars plana vitrectomy from 73 eyes of 73 patients having different RD types such as RRD without PVR (n=30), with PVR (n=16), and PDR with tractional RD (n=8), 19 eyes having ERM were used as control samples. A multiplex chemiluminescent immunoassay was performed to measure the concentrations of 48 cytokines, chemokines, and growth factors.

The expression levels of eotaxin, IFN-gamma, IL-6, IL-8, IL-16, MCP-1, MIF, and MIP-1beta were significantly higher in all groups of RD compared to the group of ERM. The levels of CTACK, IP-10, SCGF-beta, and SDF-1alpha were significantly higher in patients with diabetic tractional RD and PVR. Increased levels of VEGF and IL-18 were detected in PDR. In the subgroup analysis levels of HGF, IL-6, IL-8, IL-16, IFN-gamma, MCP-1, and MIF were significantly higher in all groups of RD compared to ERM. Levels of CTACK, eotaxin, G-CSF, IP-10, MIG, SCF, SCGF-beta, SDF-1alpha were significantly higher in PVR compared to macula on RRD and ERM. Levels of IL-1ra, IL-5, IL-9, M-CSF, MIP-1alpha, TRAIL, and IL2-Ralpha were significantly higher in PVR compared to macula on RRD.

Our results indicate that complex and significant immunological mechanisms are associated with the pathogenesis of different forms of RD: levels of selected cytokines, chemokines, and growth factors are elevated in the vitreous of eyes with RD. Furthermore, the position of macula lutea significantly influences the intravitreal cytokine expression. The detected proteins are present in different concentrations in all RD eyes. In the presence of PVR and PDR, levels of the majority of cytokines are significantly elevated, thus they may serve as biomarkers to estimate the progression or severity level of proliferation, and later to invent personalized therapeutic strategies to slow down or prevent pathological changes.

8 References

1. Wong TY, Cheung CM, Larsen M, Sharma S, Simo R. (2016) Diabetic retinopathy. Nat Rev Dis Primers, 2: 16012.
2. Pollreisz A, Sacu S, Eibenberger K, Funk M, Kivaranovic D, Zlabinger GJ, Georgopoulos M, Schmidt-Erfurth U. (2015) Extent of Detached Retina and Lens Status Influence Intravitreal Protein Expression in Rhegmatogenous Retinal Detachment. Investigative ophthalmology & visual science, 56(9): 5493-502.
3. Kaufman PL. (1976) Prognosis of primary rhegmatogenous retinal detachments. 2. Accounting for and predicting final visual acuity in surgically reattached cases. Acta Ophthalmol, 54(1): 61-74.
4. Pastor JC, Fernandez I, Rodriguez de la Rua E, Coco R, Sanabria-Ruiz Colmenares MR, Sanchez-Chicharro D, Martinho R, Ruiz Moreno J M, García Arumi J, Suárez de Figueroa M, Giraldo A, Manzanas L. (2008) Surgical outcomes for primary rhegmatogenous retinal detachments in phakic and pseudophakic patients: the Retina 1 Project--report 2. Br J Ophthalmol, 92(3): 378-82.
5. Pastor JC. (1998) Proliferative vitreoretinopathy: an overview. Survey of ophthalmology, 43(1): 3-18.
6. Chaudhary R, Scott RAH, Wallace G, Berry M, Logan A, Blanch RJ. (2020) Inflammatory and Fibrogenic Factors in Proliferative Vitreoretinopathy Development. Translational vision science & technology, 9(3): 23.
7. Garweg JG, Tappeiner C, Halberstadt M. (2013) Pathophysiology of proliferative vitreoretinopathy in retinal detachment. Survey of ophthalmology, 58(4): 321-9.
8. Glazer LC, Abrams GW. Proliferative Vitreoretinopathy. In Freeman, ed. Practical Atlas of Retinal Disease and Therapy. New York: Raven Press; 1993: 279-297.
9. The classification of retinal detachment with proliferative vitreoretinopathy. Ophthalmology. 1983;90(2):121-5.

10. Machemer R, Buettner H, Norton EW, Parel JM. (1971) Vitrectomy: a pars plana approach. Transactions - American Academy of Ophthalmology and Otolaryngology American Academy of Ophthalmology and Otolaryngology, 75(4): 813-20.
11. Kauffmann DJ, van Meurs JC, Mertens DA, Peperkamp E, Master C, Gerritsen ME. (1994) Cytokines in vitreous humor: interleukin-6 is elevated in proliferative vitreoretinopathy. Investigative ophthalmology & visual science, 35(3): 900-6.
12. Elner SG, Elner VM, Jaffe GJ, Stuart A, Kunkel SL, Strieter RM. (1995) Cytokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. Current eye research, 14(11): 1045-53.
13. Abu El-Asrar AM, Struyf S, Kangave D, Geboes K, Van Damme J. (2006) Chemokines in proliferative diabetic retinopathy and proliferative vitreoretinopathy. European cytokine network, 17(3):155-65.
14. Capeans C, De Rojas MV, Lojo S, Salorio MS. (1998) C-C chemokines in the vitreous of patients with proliferative vitreoretinopathy and proliferative diabetic retinopathy. Retina, 18(6):546-50.
15. Dai Y, Wu Z, Sheng H, Zhang Z, Yu M, Zhang Q. (2015) Identification of inflammatory mediators in patients with rhegmatogenous retinal detachment associated with choroidal detachment. Molecular vision, 21: 417-27.
16. Ricker LJ, Kessels AG, de Jager W, Hendrikse F, Kijlstra A, la Heij EC.(2012) Prediction of proliferative vitreoretinopathy after retinal detachment surgery: potential of biomarker profiling. American journal of ophthalmology, 154(2): 47-54.e2.
17. Hillon G. (1983) The classification of retinal detachment with proliferative vitreoretinopathy. Ophthalmology, 90(2): 121-5.
18. Pennock S, Kim D, Mukai S, Kuhnle M, Chun DW, Matsubara J, Cui J, Ma P, Maberley D, Samad A, Van Geest RJ, Oberstein SL, Schlingemann RO, Kazlauskas A. (2013) Ranibizumab is a potential prophylaxis for proliferative vitreoretinopathy, a nonangiogenic blinding disease. The American journal of pathology, 182(5): 1659-70.

19. Kunikata H, Yasuda M, Aizawa N, Tanaka Y, Abe T, Nakazawa T. (2013) Intraocular concentrations of cytokines and chemokines in rhegmatogenous retinal detachment and the effect of intravitreal triamcinolone acetonide. American journal of ophthalmology,155(6): 1028-37.e1.
20. Asaria RH, Kon CH, Bunce C, Charteris DG, Wong D, Khaw PT, Aylward GW. (2001) Adjuvant 5-fluorouracil and heparin prevents proliferative vitreoretinopathy : Results from a randomized, double-blind, controlled clinical trial. Ophthalmology, 108(7): 1179-83.
21. Sadaka A, Sisk RA, Osher JM, Toygar O, Duncan MK, Riemann CD. (2016) Intravitreal methotrexate infusion for proliferative vitreoretinopathy. Clinical ophthalmology, 10: 1811-7.
22. Kawahara S, Hata Y, Kita T, Arita R, Miura M, Nakao S, Mochizuki Y, Enaida H, Kagimoto T, Goto Y, Hafezi-Moghadam A, Ishibashi T. (2008) Potent inhibition of cicatricial contraction in proliferative vitreoretinal diseases by statins. Diabetes, 57(10): 2784-93.
23. Mysore Y, Del Amo EM, Loukovaara S, Hagström M, Urtti A, Kauppinen A. (2021) Statins for the prevention of proliferative vitreoretinopathy: cellular responses in cultured cells and clinical statin concentrations in the vitreous. Sci Rep. 11(1): 980.
24. Markus B, Pato Z, Sarang Z, Albert R, Tozser J, Petrovski G, Csosz E. (2017) The proteomic profile of a mouse model of proliferative vitreoretinopathy. FEBS open bio. 7(8): 1166-77.
25. Heffer AM, Wang V, Libby RT, Feldon SE, Woeller CF, Kuriyan AE. (2020) Salinomycin inhibits proliferative vitreoretinopathy formation in a mouse model. PloS one, 15(12): e0243626.
26. Khan MA, Brady CJ, Kaiser RS. (2015) Clinical management of proliferative vitreoretinopathy: an update. Retina, 35(2): 165-75.
27. Iyer SSR, Regan KA, Burnham JM, Chen CJ. (2019) Surgical management of diabetic tractional retinal detachments. Survey of ophthalmology, 64(6): 780-809.
28. Wang Y, Gao S, Zhu Y, Shen X. (2017) Elevated Activating Transcription Factor 4 and Glucose-Regulated 78 Kda Protein Levels Correlate with

Inflammatory Cytokines in the Aqueous Humor and Vitreous of Proliferative Diabetic Retinopathy. Current eye research, 42(8): 1202-8.

29. Dai Y, Wu Z, Wang F, Zhang Z, Yu M. (2014) Identification of chemokines and growth factors in proliferative diabetic retinopathy vitreous. BioMed research international, 2014: 486386.
30. Salam A, Mathew R, Sivaprasad S. (2011) Treatment of proliferative diabetic retinopathy with anti-VEGF agents. Acta ophthalmologica, 89(5): 405-11.
31. Song Z, Sun M, Zhou F, Huang F, Qu J, Chen D. (2014) Increased intravitreous interleukin-18 correlated to vascular endothelial growth factor in patients with active proliferative diabetic retinopathy. Graefe's archive for clinical and experimental ophthalmology, 252(8): 1229-34.
32. Xu Y, Cheng Q, Yang B, Yu S, Xu F, Lu L, Liang X. (2015) Increased sCD200 Levels in Vitreous of Patients With Proliferative Diabetic Retinopathy and Its Correlation With VEGF and Proinflammatory Cytokines. Investigative ophthalmology & visual science, 56(11): 6565-72.
33. Loukovaara S, Piippo N, Kinnunen K, Hytti M, Kaarniranta K, Kauppinen A. (2017) NLRP3 inflammasome activation is associated with proliferative diabetic retinopathy. Acta ophthalmologica, 95(8): 803-8.
34. Kaarniranta K, Salminen A. (2009) Age-related macular degeneration: activation of innate immunity system via pattern recognition receptors. Journal of molecular medicine, 87(2): 117-23.
35. Takahashi S, Adachi K, Suzuki Y, Maeno A, Nakazawa M. (2016) Profiles of Inflammatory Cytokines in the Vitreous Fluid from Patients with Rhegmatogenous Retinal Detachment and Their Correlations with Clinical Features. BioMed research international, 2016: 4256183.
36. Rasier R, Gormus U, Artunay O, Yuzbasioglu E, Oncel M, Bahcecioglu H. (2010) Vitreous levels of VEGF, IL-8, and TNF-alpha in retinal detachment. Current eye research, 35(6): 505-9.
37. Ricker LJ, Kijlstra A, Kessels AG, de Jager W, Liem AT, Hendrikse F, La Heij EC. (2011) Interleukin and growth factor levels in subretinal fluid in rhegmatogenous retinal detachment: a case-control study. PloS one, 6(4): e19141.

38. Wladis EJ, Falk NS, Iglesias BV, Beer PM, Gosselin EJ. (2013) Analysis of the molecular biologic milieu of the vitreous in proliferative vitreoretinopathy. Retina, 33(4):807-11.
39. Keles A, Sonmez K, Erol YO, Ayyıldız SN, Ogus E. (2021) Vitreous levels of vascular endothelial growth factor, stromal cell-derived factor-1α, and angiopoietin-like protein 2 in patients with active proliferative diabetic retinopathy. Graefe's archive for clinical and experimental ophthalmology, 259(1): 53-60.
40. Morstyn G, Brown S, Gordon M, Crawford J, Demetri G, Rich W, McGuire B, Foote M, McNiece I. (1994) Stem cell factor is a potent synergistic factor in hematopoiesis. Oncology,51(2): 205-14.
41. Hoffman R, Tong J, Brandt J, Traycoff C, Bruno E, McGuire BW, Gordon MS, McNiece I, Srour EF. (1993) The in vitro and in vivo effects of stem cell factor on human hematopoiesis. Stem Cells, 11 Suppl 2: 76-82.
42. Duarte RF, Frank DA. (2000) SCF and G-CSF lead to the synergistic induction of proliferation and gene expression through complementary signaling pathways. Blood, 96(10): 3422-30.
43. Ward SG, Westwick J. (1998) Chemokines: understanding their role in T-lymphocyte biology. Biochem J. 333 (Pt 3): 457-70.
44. Ito C, Sato H, Ando K, Watanabe S, Yoshiba F, Kishi K, Furuya A, Shitara K, Sugimoto S, Kohno H, Hiraoka A, Hotta T. (2003) Serum stem cell growth factor for monitoring hematopoietic recovery following stem cell transplantation. Bone marrow transplantation, 32(4): 391-8.
45. Yates CC, Whaley D, Hooda S, Hebda PA, Bodnar RJ, Wells A. (2009) Delayed reepithelialization and basement membrane regeneration after wounding in mice lacking CXCR3. Wound Repair Regen. 17(1): 34-41.
46. Huen AC, Wells A. (2012) The Beginning of the End: CXCR3 Signaling in Late-Stage Wound Healing. Adv Wound Care, 1(6): 244-8.
47. Cox JH, Dean RA, Roberts CR, Overall CM. (2008) Matrix metalloproteinase processing of CXCL11/I-TAC results in loss of chemoattractant activity and altered glycosaminoglycan binding. J Biol Chem.283(28): 19389-99.

48. Ridiandries A, Tan JTM, Bursill CA. (2018) The Role of Chemokines in Wound Healing. Int J Mol Sci.19(10).
49. Takai K, Hara J, Matsumoto K, Hosoi G, Osugi Y, Tawa A, Okada S, Nakamura T. (1997) Hepatocyte growth factor is constitutively produced by human bone marrow stromal cells and indirectly promotes hematopoiesis. Blood, 89(5): 1560-5.
50. Matsuda-Hashii Y, Takai K, Ohta H, Fujisaki H, Tokimasa S, Osugi Y, Ozono K, Matsumoto K, Nakamura T, Hara J. (2004) Hepatocyte growth factor plays roles in the induction and autocrine maintenance of bone marrow stromal cell IL-11, SDF-1 alpha, and stem cell factor. Exp Hematol. 32(10): 955-61.
51. Lashkari K, Rahimi N, Kazlauskas A. (1999) Hepatocyte growth factor receptor in human RPE cells: implications in proliferative vitreoretinopathy. Investigative ophthalmology & visual science, 40(1): 149-56.
52. Grierson I, Heathcote L, Hiscott P, Hogg P, Briggs M, Hagan S. (2000) Hepatocyte growth factor/scatter factor in the eye. Prog Retin Eye Res.;19(6): 779-802.
53. Josifovska N, Lumi X, Szatmari-Toth M, Kristof E, Russell G, Nagymihaly R, Anisimova N, Malyugin B, Kolko M, Ivastinović D, Petrovski G. (2019) Clinical and molecular markers in retinal detachment-From hyperreflective points to stem cells and inflammation. PloS one, 14(6): e0217548.
54. Roybal CN, Velez G, Toral MA, Tsang SH, Bassuk AG, Mahajan VB. (2018) Personalized Proteomics in Proliferative Vitreoretinopathy Implicate Hematopoietic Cell Recruitment and mTOR as a Therapeutic Target. American journal of ophthalmology, 186: 152-63.
55. Garweg JG, Zandi S, Pfister I, Rieben R, Skowronska M, Tappeiner C. (2019) Cytokine profiles of phakic and pseudophakic eyes with primary retinal detachment. Acta ophthalmologica, 97(4): e580-e8.
56. Zandi S, Pfister IB, Traine PG, Tappeiner C, Despont A, Rieben R, Skowronska M, Gaerweg JG. (2019) Biomarkers for PVR in rhegmatogenous retinal detachment. PloS one, 14(4): e0214674.

9 Bibliography of the candidate's publications

Thesis related publications

1. **Balogh A**, Milibák T, Szabó V, Nagy ZZ, Kaarniranta K, Resch MD. Immunological biomarkers of the vitreous responsible for proliferative alteration in the different forms of retinal detachment. BMC ophthalmology. 2020;20(1):491. **IF: 2,209**

2. **Balogh A**, Milibák T, Szabó V, Nagy ZZ, Resch MD. Position of macula lutea and presence of proliferative vitreoretinopathy affect vitreous cytokine expression in rhegmatogenous retinal detachment. PloS one. 2020;15(6):e0234525. **IF: 3,240**

Other publications

1. Resch MD, **Balogh A**, Deak GG, Nagy ZZ, Papp A. Vascular density in age-related macular degeneration after one year of antiVEGF treatment with treat-and-extend and fixed regimens. PloS one. 2020;15(2). **IF: 3,240**

2. Resch MD, **Balogh A**, Sándor GL, Géhl Z, Nagy ZZ. Vitrectorhexis in penetrating eye injuries in adults. European journal of ophthalmology. 2019;29(6):689-93. **IF: 1,642**

3. Resch MD, **Balogh A.** Diagnosis and treatment of retinal detachment. [A retinaleválás kórisméje és gyógyítása.] Orvostovábbképző Szemle. 2018;1218-2583 25 (11):. 35-41

4. Resch M, Barcsay G, Ecsedy M, Borbándy Á, Géhl Z, **Balogh A**, Szabó A, Nagy ZZ, Papp A. Angiography of the ocular fundus without dye: Optical coherence tomography based angiography in exsudative age-related macular degeneration. [Szemfenéki érfestés festék nélkül: Az optikai koherencia tomográfia alapú angiográfia exsudativ típusú időskori maculadegenerációban.] Orvosi Hetilap. 2016;157(42):1683-90. **IF: 0,349**

5. **Balogh A**, Rodler K., Papp A, Nagy ZZ, Resch MD. The effect of ranibizumab on central, parafoveal and perifoveal retinal thickness in exudative age-related macular degeneration. [Ranibizumab centrális, parafoveális és perifoveális retina vastagságra gyakorolt hatásának vizsgálata nedves típusú időskori makuladegenerációban.] Szemészet. 2021;158(2): 80-85.

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