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Brief Report

TITLE: Proinflammatory cytokine profile differences between primary open angle and pseudoexfoliative glaucoma

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Short Title: Pro-inflammatory cytokines as glaucoma biomarkers

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ABSTRACT:
Introduction:
Few studies have investigated glaucoma biomarkers in aqueous humor and tear and have found elevations of proinflammatory cytokines in patients with primary open-angle glaucoma (POAG) and pseudoexfoliative glaucoma (PXG). In this study we investigate differences in inflammatory cytokines between POAG and PXG patients to find specific disease biomarkers.
Methods:
For this purpose, tear and aqueous humor samples of 14 eyes with POAG and 15 eyes with PXG undergoing cataract surgery were immunoassayed for 27 pro-inflammatory cytokines. The concentrations of cytokines in tear and aqueous humor and their association with clinical variables were analysed, correlated and compared between the groups.
Results:
We found that the levels of three cytokines differed significantly in the aqueous humor of POAG and PXG patients: IL-12 and IL-13 were higher in the POAG group, while MCP-1(MCAF) was higher in the PXG group. The number of topical hypotensive medications was correlated with diminished levels of two cytokines (IL-7 and basic fibroblast growth factor) in aqueous humor in the POAG group and with diminished levels of IL-12 in tear in the PXG group.
Conclusion:
We conclude that both POAG and PXG show elevated concentrations of proinflammatory cytokines in tear and aqueous humor that could be used as biomarkers for these types of glaucoma and that the concentrations in aqueous humor of three cytokines: IL-12, IL-13 and MCP-1(MCAF) could be used to differentiate POAG and PXG.

MAIN TEXT

1. INTRODUCTION

There are various types of glaucomas that affect predominantly certain age groups, but the most common form in adults is open-angle glaucoma, which may be primary or secondary. Primary Open-Angle Glaucoma (POAG) represents 75% of all glaucomas and, although various risk factors for this type of glaucoma have been identified, its exact cause remains unknown. [1,2] The most common form of secondary open angle glaucoma is Pseudoexfoliative Glaucoma (PXG). [1] This occurs in the context of pseudoexfoliation syndrome (PXS), which is characterized by the deposition of fine fibrillary material in the anterior segment of the eye, along with other organs. [1,3]

Because POAG and PXG often course without symptoms, its diagnosis is often made when there is already extensive irreversible damage to the optic nerve. Therefore, it is important to find biomarkers of the disease that could allow an early diagnosis and deepen our knowledge of glaucoma pathogenesis. Recent studies have suggested that ischemia, oxidative stress and, especially inflammation, may be involved in PXS and POAG. [4,5] In this regard, recent studies have documented an elevation of inflammatory cytokines in POAG and PXG, with increased cytokine levels in tear of patients with POAG. [6] In addition, two earlier studies of our group revealed higher cytokine levels in tear and aqueous humor of POAG patients and specifically in the tears of patients treated with topical drugs containing preservatives. [7,8] Other authors have also found increased cytokine levels in the aqueous humor of patients with POAG [9] or in both PXG and POAG. [10,11] Finally, various studies have found differences in aqueous humor growth factors or cytokine levels, but only in patients with PXG. [12,13,14,15,16,17] Thus, although previous studies investigating cytokine levels have reported variable results in POAG and PXG, they all suggest that inflammation is involved in their pathophysiology could thus serve as a biomarker for these types of glaucoma.

The main objective of the present study was to investigate differences in inflammation between POAG and PXG (the most common forms of the disease) that could give insight in their pathogenesis. For this purpose, we determined the levels of 27 inflammatory cytokines in tear and aqueous humor of patients with POAG and PXG and analyzed the differences between these types of glaucoma.

2. METHODS

*2.1. **Patients***

This cross-sectional observational study was carried out in patients with POAG or PXG of the Glaucoma Unit of the Hospital Clinico San Carlos in Madrid (Spain) that were controlled with topical hypotensive treatment and underwent cataract surgery. The study was approved by the Clinical Research Ethics Committee of the hospital and followed the Helsinki Declaration. All the patients were informed about the procedure and signed the consent form.

Eighteen patients with POAG and 17 patients with PXG that were scheduled for cataract surgery between January and April of 2019 were included. The inclusion criteria were: age older than 40 years, diagnosis of POAG or PXG at least 2 years before and topical hypotensive treatment maintained for the last 6 months. The exclusion criteria were previous ocular surgery, YAG iridotomy, intraocular or intravitreal injections or other ocular pathology.

From the medical records of the patients, the following data was obtained: age, sex, previous diseases, surgeries and treatments, including the number and type of ocular hypotensive drugs. From the data of the visit prior to surgery, we also annotated the Best Corrected Visual Acuity (BCVA), the intraocular pressure (IOP), the Mean Deviation (MD) and Corrected Loss of Variance (CLV) of the 24-2 Visual Field (VF; Octopus, Haag Streit) and the peripapillary Nerve Fiber Layer Thickness (pNFLT; Optic Coherence Tomography, Spectralis, Heidelberg). Of the three-month follow-up, the BCVA and number of topical medications were included.

The patients were informed of the study at their arrival to the hospital for the cataract operation (between 8:30-12:30 AM). Before initiating the procedure for anesthesia or dilation of the pupil, a tear sample (3-5 µl) was obtained without anesthesia from the inferior fornix with a glass capilar (5 µL; Drummond Microcaps; Broomall, PA, USA). The aqueous humor (40-50 µL) sample was obtained through a paracentesis with a 30G needle on a syringe as the first step of the surgery. Both samples were immediately transported to the Immunology Department, where tear samples were diluted with buffered saline to give a final volume of 50µl and were then immediately frozen at -80ºC until processing.

*2.2. **Cytokine determination***

Cytokine levels in tear and aqueous humor were analyzed using the Bio-Plex Pro™ Human Cytokine 27-Plex Inmunoassay kit (Laboratorios Bio-Rad SA, Spain) according to the manufacturers intructions. This kit uses fluorescent magnetic surfaces and antibodies and allows the quantification of 27 pro-inflammatory cytokines:

Interleukin (IL)-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL9, IL-10, IL-12, IL-13, IL-15, IL-17A, eotaxin, basic Fibroblast Growth Factor (bFGF), Granulocyte Colony-Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Interferon gamma (IFN-γ), Interferon gamma-Induced Protein 10 (IP-10), Monocyte Chemoattractant Protein (Monocyte Chemotactic and Activating Factor)-1 (MCAF), Macrophage Inflammatory Proteins (MIP) forms 1α and 1β: MIP-1α, MIP-1β, platelet-derived growth factor BB dimer (PDGF)-BB, Regulated on Activation, Normal T Cell Expressed and Secreted factor (RANTES), tumor necrosis factor (TNF)-α and Vascular Endothelial Growth Factor (VEGF). For this assay, 50 µL of each sample were placed in a well and the platform was read using the Luminex MAGPIX™ (Luminex Corporation, Austin TX USA). Cytokine concentrations were determined by interpolation of the fluorescence measurements of each sample and adjusting it to their standard curves. Also, for each sample a correction factor according to the dilution was used. Finally, the software Bio-Plex Manager™ (Bio-Rad, Hercules, CA, USA) calculated the concentration of the 27 cytokines.

2.3. ***Data analysis***

The data from the medical records and cytokine concentrations was introduced in Microsoft Excel sheets and analyzed with the software SPSS (IBM SPSS Statistics for Mac 22.0 (IBM Corp., Armonk, NY, USA). Data is expressed as means ± standard deviation (SD). Quantitative variables were compared using the t-test or the Mann–Whitney test depending on the normality of the data. The correlation between cytokine levels in tear and aqueous humor and number of treatments was analyzed using the Spearman's Rho correlation coefficient. A p value <0.05 was considered statistically significant.

3. RESULTS

3.1. ***Patients***

Although 18 eyes of patients with POAG and 17 eyes of patients with PXG were initially included in the study, some samples had to be discarded because of insufficient quantity of the sample and finally only 14 eyes with POAG and 15 eyes with PXG could be included.

Table 1 depicts the characteristics of both study groups. Mean age of the patients was similar in both groups. BCVA increased after cataract surgery in both groups, and was significantly higher in the POAG than in the PXG group both at baseline and postoperatively. The number of topical hypotensive drugs used preoperatively and postoperatively was similar in both groups and all patients had been on that treatment for at least 6 months. Preoperative and postoperative IOP was also similar in both groups.

The MD of the VF was -6.49 ± 6,28 and -10.45 ± 6.35 dB in the POAG and PXG group, respectively, with significant differences between them (Table 1). The global and sectorial pRNFL thicknesses were similar between groups, except for the superotemporal sector that was significantly thinner in the PXG group (Table 1).

2.2. ***Cytokine concentrations in tear and aqueous humor***

Cytokine concentrations (pg/mL) can be observed in Figures 1 and 2 and Table 2. Cytokine concentrations varied greatly in aqueous humor and tear in both experimental groups. However, when cytokine concentrations in tear or aqueous humor were compared between groups, three cytokines showed significant differences: IL-12 and IL-13 were significantly higher in the POAG group and MCP-1(MCAF) was significantly increased in PXG patients (Figures 1,2; Table 2).

2.3. ***Correlation between cytokine concentrations in tear and aqueous humor***

There were no significant correlations between the cytokine concentrations in tear and aqueous humor in the POAG group (Table 2), but there were significant correlations in the concentrations of three cytokines in the PXG group: IFN-γ, MIP-1β and VEGF (Table 2).

2.4. ***Correlation between cytokine concentration and number of topical treatments***

There was a significant negative correlation between the number of topical treatments used at baseline and the concentrations of two cytokines in POAG patients: IL-7 and bFGF in aqueous humor (Table 2). There was also a significant negative correlation between the number of topical treatments used at baseline and the concentration of IL-12 (Table 2) in tear in the PXG group.

4. DISCUSSION

In the present study we have used a sensitive immunoassay to analyze the concentrations of 27 inflammatory cytokines in tear and aqueous humor of patients with POAG or PXG that were undergoing cataract surgery to discern whether these cytokines were involved differently in these types of glaucoma and could therefore be used as biomarkers.

We documented great variations of cytokine concentrations in both groups of patients, both in tear and aqueous humor. This indicates a great variability of cytokine expression between patients even within a diagnostic group,

and it has been documented previously in normal patients and in patients with different pathologies such as Sjögren syndrome or glaucoma. [6,7,8,18,19,20]
Our results document a tendency for the cytokines to show a high or low concentration both in tear and in aqueous humor. However, we could not document a significant correlation between the cytokine concentrations in tear and aqueous humor in the POAG group and we could only document a significant correlation in the concentrations of three cytokines in the PXG group: IFN Gamma, MIP-1β and VEGF. When comparing cytokine concentrations in tear and aqueous humor, other authors have found higher levels of cytokines in tears than in the aqueous humor [20], but in this study and in a previous study of our group we have failed to do so. [8]
Our main objective was to add insight into the pathogenesis of POAG and PXG and for this purpose we have analysed the levels of inflammatory cytokines in these two types of glaucoma and the differences between them. We have found high levels of four cytokines: IL-1RA, IL-15, IP-10 and VEGF both in tear and aqueous humor of POAG and PXG patients. Elevated levels of some of these cytokines in open angle glaucoma patients when compared to the control group have also been observed in previous works of our group [7,8], and increased levels of VEGF, TNF-α and IL-6 have also been previously documented in tear of POAG patients. [6] It has been proposed that elevated levels of VEGF and TGF-β in the aqueous humor could be responsible for trabeculectomy failure in POAG patients [21], but other authors have argued that only diminished levels of IFN-γ, GM-CSF and IL-5 in tear are associated with trabeculectomy complications. [20]
When we compared cytokine concentrations in tear and aqueous humor of POAG and PXG patients, we found statistically significant differences only in the aqueous humor. IL-12 and IL-13 were significantly higher in the POAG group, while MCP-1 (MCAF) was significantly increased in the PXG group. Thus, these cytokines could be used as aqueous humor biomarkers for these types of glaucoma and may be involved in the different pathogenesis of each type of glaucoma. IL-12 is secreted by macrophages and dendritic cells. It acts by activating NK cells and promoting Th1 differentiation, as well as stimulating IFN-γ synthesis. Therefore, despite not being a commonly tested cytokine, IL-12 could serve as a marker for diagnosis and be specifically involved in POAG pathogenesis. However, the pleiotropic characteristics of cytokines render an exact assignment difficult. In accordance with this, previous studies of our group have also found increased levels of IL-12 in tear and aqueous humor of POAG patients and increased levels of IL-12 and IL-13 in patients with glaucoma treated with preserved latanoprost. [7,8] Cytokine elevations in the aqueous humor may depend on the type of glaucoma. In this regard, Duvesh et al. found that IL-8, eotaxin and IP-10 are significantly increased in closed-angle glaucoma [22], others revealing differences in IL-6, IL-8, G-CSF, MCP-1, MCP-3, and VEGF. [23,24]
Our results document increased MCP-1 (MCAF) concentrations in aqueous humor of the PXG group when compared to the POAG group and may reflect an increased inflammation in the PXG group. MCP-1 (MCAF), also known as CCL2, is a potent chemotactic factor for monocytes and macrophages and plays a role in various inflammatory diseases and probably in conjunctival scarring. [25,26] Notwithstanding, the role of this cytokine in PXG pathogenesis is still unclear. Given that the blood-aqueous barrier breakdown in PXG patients results in the entry of inflammatory cytokines and extracellular matrix material into the anterior chamber, the differences in cytokine levels could be due to this blood-aqueous breakdown rather than inflammation. [27] Although in our work MCP-1 (MCAF) is differentially elevated in PXG, it has been reported to be elevated in the aqueous humor in both POAG and PXG.[11] PXG is characterized by rapid evolution and severe prognosis and other cytokines including IL-6, IL-17 [14], TNF-α [17], TGF-β, PDGF and IL-8 [15] have been described to be elevated in the aqueous humor of PXG.
All the patients included in our POAG and PXG groups had cataracts and glaucoma and were receiving topical hypotensive medication (with and without preservatives) and this may have influenced the cytokine profiles. It is not known how cataracts affect cytokine concentrations in the aqueous humor, but it has been documented that ocular hypotensive treatments with preservatives influence the tear cytokine profile. [6,7] As most patients in both groups were using more than one drug, it was not possible to correlate cytokine levels and the type of drug. However, all patients using one drug were on prostaglandins, and patients with two or more drugs were also on beta-blockers and/or carbonic anhydrase inhibitors. We analyzed if cytokine concentrations were correlated to the number of ocular hypotensive drugs medications that the patients were receiving. We found that the number of ocular hypotensive medications produced a decrease of the IL-7 and bFGF concentrations in aqueous humor of the POAG group, and of the IL-12 concentrations in tear of the PXG group. Thus, the topical treatment may have decreased inflammation and influenced in part the results.
Although more patients were initially included in the study, the samples of only 14 eyes with POAG and 15 eyes with PXG could be analyzed. The composition of the groups was generally comparable: at the baseline visit, age,

IOP, global thickness of the pRNFL and number of topical hypotensive medications was similar in both groups. Nevertheless, the eyes included in the PXG group in this study could have more advanced cataracts and increased functional and structural glaucoma damage, or different disease timeline and /or different previous treatments. Indeed, the MD of the VF was significantly more negative in the PXG group, the BCVA was lower in the PXG, both at baseline and postoperatively, and the thickness of the superotemporal sector of the pRNFL was also significantly thinner in this group. However, we believe that the elevations in cytokine concentration in the tear aqueous humor both in POAG and PXG and the differences in cytokine concentrations that we find between the groups cannot be solely explained on the basis of different severity of glaucoma damage.
Finally, although further studies are needed to define the concentrations of cytokines in tear and aqueous humor of POAG and PXG patients and their variations with the clinical course and severity of the disease, it is possible that these could be used in the future as non invasive (in the case of tears) and rapid methods to diagnose and grade these diseases.

5. Conclusions

In conclusion, the analysis of 27 inflammatory cytokines in tear and aqueous humor of POAG and PXG patients has allowed us to document that the levels of three cytokines in the aqueous humor differed between the groups: IL-12 and IL-13 were significantly higher in the POAG group, and MCP-1(MCAF) was significantly higher in the PXG group. Thus, although further studies are needed, these cytokines could be particularly involved in the pathogenesis of these forms of glaucoma.

STATEMENTS

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STATEMENT OF ETHICS

The study was conducted according to the guidelines of the World Medical Association Declaration of Helsinki, and approved by the Ethics Committee of Hospital Clinico San Carlos (protocol code 18/255-E in December 2018). Written informed consent was obtained from all subjects involved in the study.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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AUTHOR CONTRIBUTIONS: Conceptualization: JMMC and JGF; methodology: JMMC, JLS, LEP; software: JLS, LEP; validation: JMMC, JGF, JFV; formal analysis: BVV, BBB; investigation: BVV, BBB, VAG; resources: VAG, JLS, LEP; data curation: BVV, BBB, JMMC, JLS, LEP; writing: original draft preparation: BVV, BBB; writing: review and editing: BVV, BBB, JMMC; visualization: JFV, JGF; supervisión: JFV,JMMC,JGF; project administration: JMMC, JGF; funding acquisition: JMMC, JGF. All authors have read and approved the final manuscript and agreed to publish the manuscript.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this article or its supplementary material files. Further enquiries can be directed to the corresponding author.

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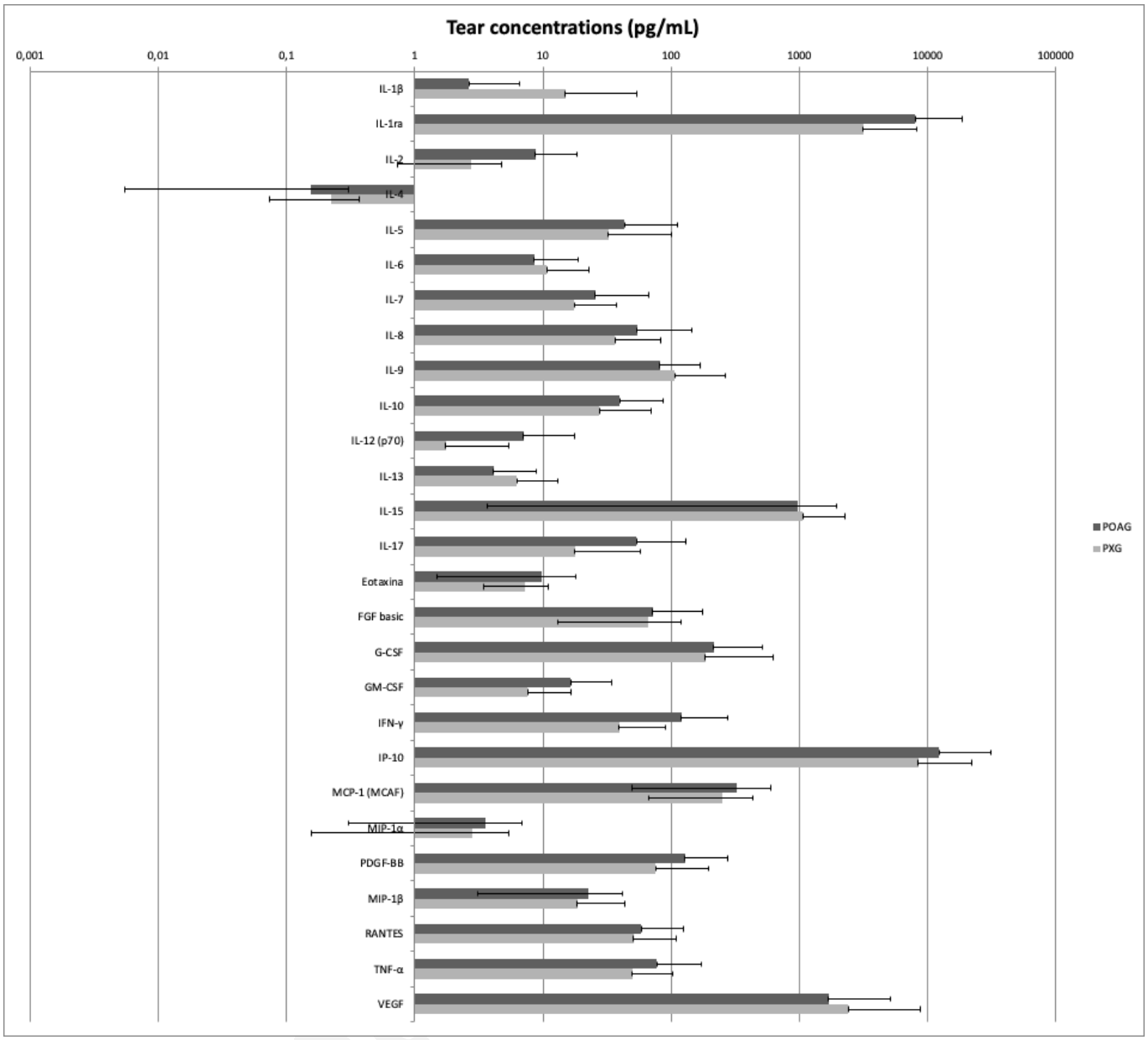
LEGENDS FOR FIGURES

Figure 1. Cytokine concentrations (pg/mL±SD) in tear in POAG and PXG. X axis: logarithmic scale.

Figure 2. Cytokine concentrations (pg/mL±SD) in aqueous humor in POAG and PXG. X axis: logarithmic scale.


Tear concentrations (pg/mL)
0,001
0,01
0,1
1
10
100
1000
10000
100000
IL-1β
IL-1ra
IL-2
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-12 (p70)
IL-13
IL-15
IL-17
Eotaxina
FGF basic
G-CSF
GM-CSF
IFN-γ
IP-10
MCP-1 (MCAF)
MIP-1α
PDGF-BB
MIP-1β
RANTES
TNF-α
VEGF
POAG
PXG

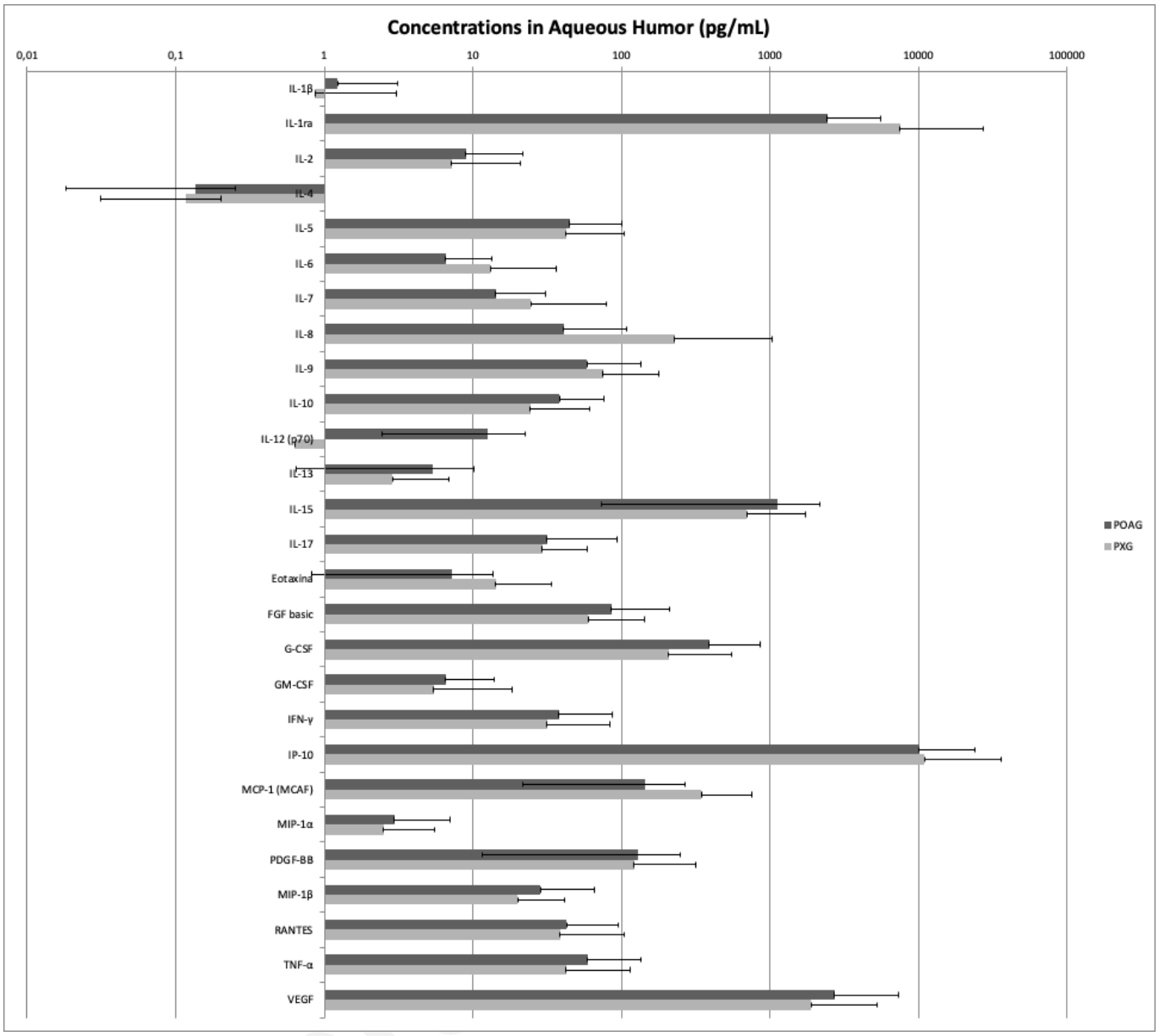

Concentrations in Aqueous Humor (pg/mL)
0,01
0,1
1
10
100
1000
10000
100000
IL-1β
IL-1ra
IL-2
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-12 (p70)
IL-13
IL-15
IL-17
Eotaxina
FGF basic
G-CSF
GM-CSF
IFN-γ
IP-10
MCP-1 (MCAF)
MIP-1α
PDGF-BB
MIP-1β
RANTES
TNF-α
VEGF
POAG
PXG

TABLE 1. DEMOGRAPHIC AND CLINICAL VARIABLES AND COMPARISONS BETWEEN GROUPS

Variable	Diagnosis		Statistical comparison (p= test)
	POAG	PXG	
Age (Mean±SD)	76.14±6.89	77.40±9.45	0.652 Mann-Whitney
Baseline BCVA	0.43±0.13	0.29±0.15	0.019 Mann-Whitney
Postoperative BCVA	0.85±0.12	0.61±0.32	0.019 Mann-Whitney
n of topical hypotensive drugs (baseline)	1.69±0.63	1.64±0.84	0.864 t-test
n of topical hypotensive drugs (postoperative)	0.69±0.75	0.79±0.80	0.757 t-test
IOP (baseline)	19.08±3.45	19.79±2.75	0.488 Mann-Whitney
IOP (postoperative)	16.54±1.81	16.71±4.08	0.756 Mann-Whitney
MD of the VF	6.49±6.28	10.45±6.35	0.020 Mann-Whitney
CLV of the VF	3.98±1.41	4.93±1.41	0.102 Mann-Whitney
pRNFL thickness			
GLOBAL	83.43±20.88	70.93±18.56	0.085 Mann-Whitney
Nasal	59.07±19.29	57.73±18.51	0.813 Mann-Whitney
Nasal Superior	93.64±25.32	78.20±29.96	0.158 Mann-Whitney
Temporal Superior	116.50±32.38	86.53±32.52	**0.023 Mann-Whitney**
Temporal	66±17.66	58.07±13.35	0.158 Mann-Whitney
Temporal Inferior	119.29±45.80	91.87±32.08	0.057 Mann-Whitney
Nasal Inferior	87.36±27.99	79.80±22.89	0.146 Mann-Whitney

LEGENDS FOR TABLES

Table 1. Demographic and clinical variables and comparisons between groups. Postoperative values were obtained 3 months after cataract surgery. The value in bold indicates a significative difference between the groups. n = number.

TABLE 2. CYTOKINE CONCENTRATIONS (pg/mL) IN TEAR AND AQUEOUS HUMOR, COMPARISON AND CORRELATIONS BETWEEN THEM AND WITH NUMBER OF TOPICAL TREATMENTS

CYTOKINE	Diagnosis										
		TEAR		COMPARISON POAG/PXG (tear)	AQUEOUS HUMOUR		COMPARISON POAG/PXG (Aqueous humor)	CORRELATION TEAR/AQUEOUS	CORRELATION WITH NUMBER OF TREATMENTS (Spearman, p=)	
		Mean	SD	Mann-Whitney p=	Mean	SD	Mann-Whitney p=	Pearson	Tear	Aqueous humor
IL-1β	POAG	2.66	3.87	1	1.23	1.90	0.425	0.522	0.159	-0.151
	PXG	14.95	39.43		0.86	2.22		-0.03	-0.174	-0.083
IL-1ra	POAG	8005.29	10535.04	0.847	2425.90	3085.20	0.78	0.481	0.021	-0.206
	PXG	3148.25	4993.16		7506.10	19693.08		-0.169	-0.347	-0.166
IL-2	POAG	8.73	9.52	0.425	8.96	12.86	0.715	0.325	-0.243	-0.475
	PXG	2.75	2.01		7.16	13.93		0.487	-0.107	-0.347
IL-4	POAG	0.15	0.15	0.123	0.14	0.12	0.715	0.134	-0.188	0.092
	PXG	0.22	0.15		0.12	0.09		0.148	0.209	-0.010
IL-5	POAG	43.41	68.39	0.683	45.00	56.29	0.505	0.522	0.216	-0.235
	PXG	32.44	68.14		42.33	62.52		0.399	-0.406	0.023
IL-6	POAG	8.52	10.31	0.377	6.54	6.91	1	0.484	-0.009	-0.141
	PXG	10.68	12.10		13.13	23.28		0.148	0.021	0.022

IL-7	POAG	25.67	41.08	0.88	14.30	16.63	0.78	0.317	0.101	**-0.659 p=0.014**
	PXG	17.52	19.76		24.50	54.87		0.289	0.009	-0.123
IL-8	POAG	53.97	91.34	0.747	40.73	66.66	1	0.163	-0.343	-0.446
	PXG	36.25	46.18		228.86	801.84		0.158	-0.285	-0.202
IL-9	POAG	81.29	86.32	0.561	58.53	76.23	0.813	0.089	-0.040	0.028
	PXG	106.63	157.65		75.42	101.62		0.251	-0.308	-0.198
IL-10	POAG	39.86	45.95	0.451	38.18	38.33	0.186	0.353	-0.117	-0.223
	PXG	27.43	42.48		24.26	37.05		0.429	-0.444	-0.059
IL-12 (p70)	POAG	7.03	10.64	0.172	12.52	10.07	**0.000**	0.293	-0.167	-0.249
	PXG	1.75	3.62		0.63	0		0.473	**-0.619 p=0.018**	-0.214
IL-13	POAG	4.10	4.74	0.621	5.37	4.73	**0.037**	-0.44	0.008	-0.452
	PXG	6.29	6.83		2.89	3.99		0.236	-0.355	0.09
IL-15	POAG	970.05	966.32	0.847	1115.45	1041.91	0.252	-0.279	-0.125	-0.159
	PXG	1058.39	1199.51		697.87	1041.52		0.314	-0.37	0.054
IL-17	POAG	53.63	77.02	0.134	31.48	62.03	0.425	-0.267	0.194	-0.483
	PXG	17.76	40.26		29.42	29.60		0.355	-0.362	0.205
Eotaxina	POAG	9.80	8.30	0.4	7.20	6.37	0.201	-0.077	-0.017	-0.156

	PXG	7.19	3.71		14.28	19.68		0.192	0.04	-0.166
FGF basic	POAG	71.38	103.32	0.477	85.91	123.26	0.715	-0.149	0.209	**-0.661 p=0.014**
	PXG	66.56	53.55		59.65	83.98		0.25	-0.322	-0.146
G-CSF	POAG	215.21	300.05	0.477	388.29	471.57	0.29	-0.444	0.041	-0.023
	PXG	184.83	435.76		207.27	341.44		0.449	-0.286	-0.201
GM-CSF	POAG	16.41	18.15	0.377	6.57	7.28	0.252	-0.487	-0.028	-0.075
	PXG	7.63	8.98		5.46	12.88		-0.079	-0.432	0.207
IFN-γ	POAG	119.38	159.26	0.270	37.87	49.23	0.621	-0.195	-0.052	-0.295
	PXG	39.50	51.02		31.58	52.63		**0.792 p<0.001**	-0.355	-0.182
IP-10	POAG	12235.07	19101.59	0.451	10045.68	13887.78	0.914	-0.268	-0.141	-0.490
	PXG	8429.80	13399.64		10981.94	24934.31		-0.296	-0.284	-0.143
MCP-1 (MCAF)	POAG	324.43	274.71	0.561	144.12	122.24	**0.014**	-0.137	-0.253	0.058
	PXG	251.16	184.59		345.76	407.48		0.255	0.061	-0.239
MIP-1α	POAG	3.55	3.24	0.591	2.96	4.12	0.88	0.423	-0.160	-0.435
	PXG	2.79	2.63		2.50	3.00		0.005	-0.434	0.016
PDGF-BB	POAG	127.72	148.31	0.561	129.87	118.31	0.290	-0.502	-0.125	-0.163
	PXG	75.50	122.13		121.57	195.56		0.269	-0.274	-0.040

MIP-1β	PO AG	2 2.53	1 9.45	0.4	2 8.39	3 7.07	0.949	0.061	-0.064	-0.291
	PX G	1 8.48	2 5.47		1 9.99	2 1.38		**0.750 p<0.001**	-0.282	-0.311
RANTES	PO AG	5 8.31	6 7.40	0.88	4 2.74	5 2.28	0.331	-0.483	-0.067	-0.243
	PX G	5 1.03	5 9.56		3 8.70	6 4.28		0.356	-0.219	-0.018
TNF-α	PO AG	7 6.93	9 3.50	0.813	5 9.33	7 6.93	0.505	-0.463	-0.189	-0.441
	PX G	4 9.66	5 2.29		4 2.53	7 1.63		0.168	-0.097	-0.129
VEGF	PO AG	1 681.76	3 448.88	1	2 713.41	4 551.83	0.813	-0.462	0.151	-0.160
	PX G	2 390.89	6 381.50		1 900.25	3 338.63		**0.621 p=0.014**	-0.152	-0.083

LEGENDS FOR TABLES

Table 2. Cytokine concentrations (pg/ml) in tear and aqueous humor, comparison and correlations between them and with number of topical treatments. Values in bold indicate a significative difference or a significant correlation between the groups.