Elevated Inflammatory Cytokines Persist in the Aqueous Humor Years After Cataract Surgery

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Purpose. There is currently limited information regarding inflammation and cytokine levels in the aqueous humor (AH) of adult patients with cataract who have undergone phacoemulsification cataract extraction without other ocular comorbidities.

Methods. AH samples were collected from healthy, non-surgical donors and donors with a history of cataract surgery performed 3 to 12 years prior. Sixty-three cytokines and growth factors were measured using bead-based ProcartaPlex immunoassays. Data analysis included normal distribution assessment, pairwise correlation, logistic regression, and ridge regression.

Results. Of the 63 molecules analyzed, 34 were selected for further study. Cytokines, such as CD40L, IL-7, MIP-1α, and LIF, were found at significantly higher concentrations in AH samples from donors with a history of cataract surgery compared with non-cataract controls. In contrast, lower concentrations of IL-23, TRAIL, IL-12p70, IFNγ, MIP-3α, and SCF were observed in post-surgical samples. Pairwise correlation analysis identified clusters of significantly correlated molecules, suggesting their potential involvement in the inflammatory environment of AH post-cataract surgery. AH concentration of 34 proteins was combined into a post-cataract surgery inflammation index (PCSII) using ridge regression, which differs significantly between post-cataract surgery donors and non-cataract controls. This PCSII shows that any increase in AH levels of these molecules can stratify cataract surgery donors into low and high-risk of inflammatory groups.

Conclusions. This study indicates that cataract surgery may lead to a chronic inflammatory state in the AH, which can persist for extended periods post-surgery.

Keywords: cataract surgery, aqueous humor (AH), growth factors, cytokines, chronic inflammation

Cataracts are a leading cause of blindness worldwide. According to the World Health Organization (WHO), approximately 65 million people are affected by cataracts, and this number is continuously increasing due to the growing aging population, with age-related cataracts being the dominant type. Cataract surgery with intraocular lens (IOL) implantation is currently the only treatment to restore vision, with an estimated 4 million surgeries performed in the United States and 26 million worldwide each year.[1,2] Despite the overall success of this surgical treatment, it carries a risk of various complications.

The most common complication is posterior capsule opacification (PCO), which occurs in 20% to 40% of adult patients within 2 to 5 years post-surgery[3] and nearly 100% in infants and children within 3 years.[4] PCO is thought to result from the accumulation of proliferated and differentiated lens epithelial cells (LECs) left behind after surgery in the posterior capsule. However, the fundamental mechanisms underlying the initiation and progression of PCO remain unclear.

Increasing clinical evidence also suggests that cataract surgery can negatively impact other ocular conditions, particularly pre-existing ones.[5,6] For example, a meta-analysis of 9 studies on cataract and dry eye disease (DED) found that over 37% of patients without pre-existing DED developed DED after cataract surgery.[7] Additionally, cataract surgery can significantly exacerbate DED in patients with pre-existing DED.[8] A study found that childhood cataract surgery is associated with a high risk of late-onset glaucoma.[9] Cataract surgery has also been shown to significantly worsen diabetic retinopathy (DR) and may even increase the likelihood of retinopathy in patients without prior retinopathy.[6,10] Other complications have also been reported, such as endophthalmitis, retinal detachment, cystoid macular edema, and iritis.[1,11,12]

Inflammation resulting from cataract surgery-induced traumatic injury and a compromised blood-aqueous barrier (BAB) is a key factor in postoperative complications. During the first few days after surgery, the wound-healing response triggers the secretion of various growth factors and inflammatory cytokines.[13–18] Notably, cytokines such as TNFα, monocyte chemoattractant protein-1 (MCP-1), and multiple interleukins (ILs) typically peak within 24 hours postoperatively.[17,18] For example, IL-6 levels can increase by over 4000-fold in the aqueous humor (AH) of patients undergoing cataract surgery.[16] Acute inflammation can lead to complications such as pain and discomfort, endophthalmitis, lens-induced uveitis, and cystoid macular edema (CME).[19,20] The AH flare, measured using laser flare and cell photometry (LFCP), is often utilized clinically to monitor inflammation and predict the risk of CME development postoperatively.[21] By the end of the initial healing phase (approximately 2–3 weeks), levels of inflammatory cytokines and growth factors typically decline. However, the long-term status of cytokine levels following surgery remains poorly understood.

Acute inflammation after surgery is well-recognized and effectively managed through advancements in surgical techniques and anti-inflammatory drug treatments.[19,22] However, whether cataract surgery induces long-term inflammation remains a subject of debate. Some studies suggest that the BAB can be restored within 3 months after surgery,[23,24] whereas others indicate the persistence of chronic inflammation in the AH postoperatively.[25] For instance, a study on congenital cataracts in children found elevated levels of IL-6, IP-10, MCP-1, and IL-2 up to 9 to 60 months after the initial surgery.[25] Similarly, Gu et al.[26] reported increased levels of cytokines, such as IL-6 and IL-8, in patients who underwent vitrectomy 6 to 80 months after cataract surgery. Using a laser flare-cell meter to monitor AH flare, Schauersberger et al.[27] observed significantly elevated inflammation in postoperative patients compared with their preoperative levels, between 12 and 35 months after surgery.

Currently, there is limited information on the status of inflammation and cytokine levels in adult patients with cataract who have undergone phacoemulsification cataract extraction a long time after surgery. In the present study, we used a multiplex immune assay to monitor 63 cytokines and growth factors in the AH of non-cataract donors and those who had cataract surgery 3 to 12 years earlier. To the best of our knowledge, this is the first study to document a comprehensive panel of cytokine levels more than 3 years after cataract surgery.

Materials and Methods

Human Aqueous Humor Collection

The AH samples were obtained from the Georgia Eye Bank and the Duke Eye Bank. AH samples from donors with no history of cataract surgery, ocular diseases, and diabetes were designated as the non-cataract (control) group. AH samples from donors who had undergone phacoemulsification cataract extraction without other ocular disorders or diabetes were designated as the post-cataract surgery group. All AH samples from donors, regardless of cataract surgery history, were collected from donor eye globes within 18 to 36 hours postmortem. A total volume of 100 to 200 µL of AH was obtained. The collected AH samples were centrifuged at 10,000 × g for 10 minutes, after which the supernatant was transferred into new Eppendorf tubes, aliquoted, and stored at −80°C until further use. The samples were only allowed to thaw once.

Multiplex Measurement of Inflammatory Molecules

We measured 63 molecules (Supplementary Table S1) in AH using bead-based ProcartaPlex immunoassays for these proteins (EPXTL650-10065-901; Thermo, Carlsbad, CA, USA). Multiplex assays were performed according to the instructions provided in the kit. Briefly, AH samples (1:5 dilution) were incubated with antibody-coated microspheres, followed by biotinylated detection antibody. Detection of the proteins was accomplished by incubation with phycoerythrin-labeled streptavidin. The resultant bead immuno-complexes were then read on a FLEXMAP3D (Luminex, Austin, TX, USA) with the instrument settings recommended by the manufacturer.

The captured median fluorescence intensity (MFI) data were then processed through Procartaplex analysis software available at https://apps.thermofisher.com/apps/procartaplex/#./. A standard curve of known concentration was also run to convert MFI of measured AH into concentration values. Both the standard concentration and its MFI values were log-transformed prior to fitting a four-parameter logistic regression. Concentrations were estimated for samples using the regression fit parameters. The resulting log-transformed concentrations were then back-transformed to concentration values. The ProcartaPlex application marked any concentration value below the lowest limit of quantitation (LLOQ) as <OOR and those above the highest concentration as OOR< (see Supplementary Table S1). Before any analysis, quality control was performed to identify the percentage of missing data, proteins with more than 10% missing data were excluded from statistical analysis. To eliminate NA's in the selected molecules, the <OOR values were replaced with the lowest standard curve values.

Statistical Analysis

Data are presented as count, and percentages for categorical variable. Normally distributed variables are presented as means ± standard deviation. The median and range are presented for non-normal variables. Before any statistical analysis, protein concentration values were log2 transformed to follow the normal distribution. After the creation of figures, the data were back transformed to natural units. Univariate differences between post-cataract surgery and non-surgery individuals were examined using boxplots and *t*-tests.

Before performing pairwise correlations, logistic regression, Kmeans, and principal component analysis (PCA), the data was log2 transformed, centered, and scaled. Pairwise correlations between individual proteins were determined using the Pearson correlation coefficient and presented as a heatmap with hierarchical clustering. Association analysis was performed by logistic regression using the "glmnet" package. The relationship between post-cataract surgery and non-cataract subjects was evaluated using stepwise logistic regression with surgery/non-surgery status incorporated as a dependent variable. Age and sex were included in separate models to adjust for these as covariates.

AH molecule levels were divided into 3 tertiles each containing 33% of the samples, the cutoff levels from the

cataract surgery group were used to count healthy and post-cataract surgery samples in each tertile. The first tertile was used as a reference to which the second and third tertiles were compared to get the odds ratio (OR). Pearson's Chi-squared test with Yates" continuity correction was used to calculate the ORs. The chi-squared test for trend in proportions was used to calculate the P value of an overall trend. Concentration values for 34 proteins were subjected to ridge regression to create a post-cataract surgery inflammation index (PCSII), as described in the study by Purohit et al.[28] The score was used for calculating OR of post-cataract surgery for the upper two tertiles using the first tertile as a reference.

All P values presented are 2-sided and a $P < 0.05$ was considered significant, when appropriate, P values were corrected for multiple testing as per Benjamini and Hochberg's method.[29] Statistical analyses were performed using the R language and environment for statistical computing (version 4.0.3; R Foundation for Statistical Computing; www.r-project.org).

Results

Description of the Samples

The AH proteins were measured in a total of 96 samples obtained from 48 individuals, consisting of 50 samples (25 individuals) from non-cataract and 46 samples (23 individuals) from post-surgery cataract donors. All donors with a history of cataract surgery underwent bilateral cataract procedures, whereas the donors without a cataract history had bilateral phakic lenses. These 96 samples were further divided by sex, into male subjects ($n = 25$) and female subjects ($n = 23$). Individuals in the post-cataract group were older, with an average age of 78.9 years, compared to 67.5 years for non-cataract individuals. After stratification by post-cataract surgery, no significant differences were observed in age or sex. The average time since cataract surgery among donors in the post-cataract group was 7.8 years, with surgeries performed 3 to 12 years before sample collection (Table 1).

Significant Differences in Multiple Cytokines Are Observed in AH Between Post-Cataract Surgery Patients and Non-Surgery Controls

After the ascertaining of missing values (see Supplementary Table S1) we selected 34 molecules for further study. Ten out of 34 molecules showed significant univariate differences based on means and t-tests (Table 2, Supplementary Table S2). The distribution of concentrations in the non-cataract and post-surgery cataract donors is presented in Figure 1 and Supplementary Figure S1. Significantly elevated proteins were CD40 ligand (CD40L; fold change [FC] = 1.29, $P = 0.043$), interleukin 7 (IL-7; FC = 1.3, $P = 0.015$), macrophage inhibitory protein-1 alpha (MIP-1α; FC = 1.39, $P = 0.049$), and leukemia inhibitory factor (LIF; FC = 1.61, $P = 0.036$) in post-cataract surgery donors. Both IL-6 and MCP-1 showed an increasing trend in post-cataract surgery donors compared with non-surgery controls, however, this increase was not statistically significant (see Fig. 1). Concentrations of IL-23 (FC = 0.44, $P = 0.027$), TRAIL (FC = 0.59, $P = 0.046$), IL12p70 (FC = 0.66, $P = 0.013$), IFNγ (FC = 0.7, $P = 0.02$), MIP-3α (FC = 0.71, $P = 0.049$), and SCF (FC = 0.86, $P = 0.019$) were lower in the samples from post-surgery patients with cataract (see Fig. 1, Table 2).

Correlation Analysis Indicated Elevated Inflammation in Post-Cataract Surgery Donors

We evaluated the correlation between the concentrations of these inflammatory proteins using Pearson's correlation analysis for the healthy and post-cataract surgery

Table 1. Donor Demographics

Parameters	Non-Cataract	Post-Surgery	Total	*P* Value
Number of donors (eyes)	25 (50)	23 (46)	48 (96)	
Sex, *n* (%)				
M	14	11	25	0.8643*
F	11	12	23	
Age, mean	67.5 ± 10.9	78.9 ± 8.2		
Years after cataract surgery, mean		7.8 ± 3.03		

Table 2. Key Cytokines Show Difference Between Healthy and Post-Cataract Surgery

Protein	Non-Cataract* ($n = 50$)	Cataract Surgery* ($n = 46$)	Fold Change	*P* Value
IL-23	6.03 ± 2.32	4.83 ± 2.27	0.44	0.0269
TRAIL	3.49 ± 1.98	2.74 ± 1.78	0.59	0.0459
IL12p70	2.70 ± 0.92	2.11 ± 0.95	0.66	0.0127
IFNγ	5.00 ± 1.08	4.49 ± 1.05	0.70	0.0198
MIP-3α	5.37 ± 1.03	4.88 ± 1.03	0.71	0.0494
SCF	4.72 ± 0.77	4.49 ± 0.83	0.86	0.0187
CD40L	5.05 ± 1.30	5.42 ± 1.18	1.29	0.0426
IL-7	2.66 ± 0.57	3.04 ± 0.58	1.30	0.0152
MIP-1α	3.06 ± 0.71	3.53 ± 1.16	1.39	0.0495
LIF	4.30 ± 0.79	4.99 ± 1.51	1.61	0.000362
IL-6	8.40 ± 2.33	8.85 ± 2.91	1.37	0.12
MCP-1	11.54 ± 1.18	11.83 ± 1.02	1.22	0.07

* Log2 transformed concentration (original unit = pg/mL).

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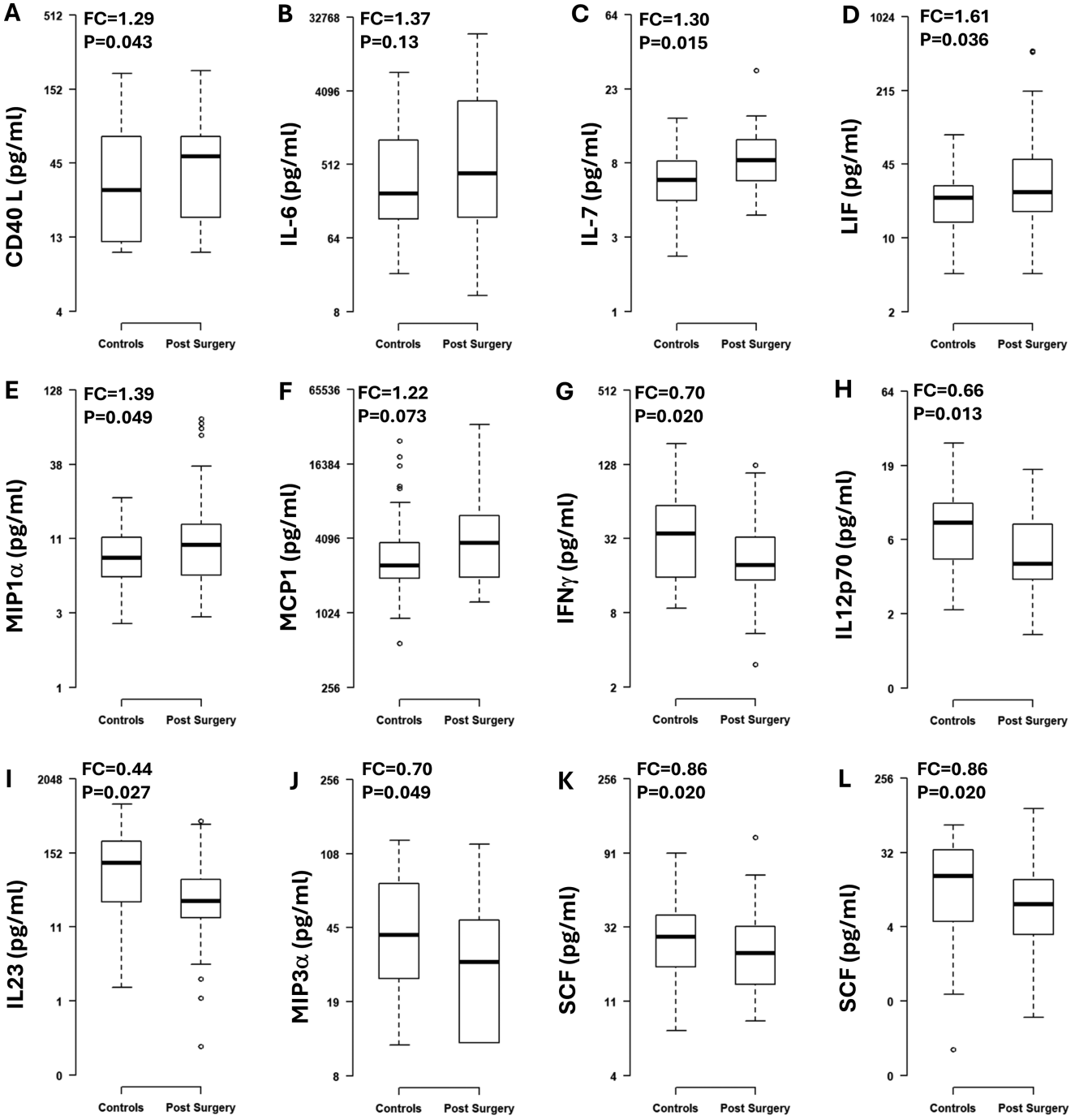

Figure 1. The distribution of concentrations of AH cytokines in AH from post-surgery cataract donors and non-cataract controls. Univariate differences between post-cataract surgery and non-cataract individuals were examined using boxplots and *t*-tests. FC, fold change. Only $P < 0.05$ is considered significant. (**A**) CD40L, (**B**) IL-6, (**C**) IL-7, (**D**) LIF, (**E**) MIP1α, (**F**) MCP1, (**G**) IFNγ, (**H**) IL12p70, (**I**) IL-23, (**J**) MIP3α, (**K**) SCF), and (**L**) TRAIL.

group separately. The correlation coefficient (r) values were subjected to hierarchical clustering to identify relationships (Fig. 2). In the heatmap of correlations from the non-cataract group, we found 2 clusters containing 23 proteins in cluster 1 and 19 proteins in cluster 2. A strong correlation ($r = 0.31$–0.91, $P < 003$) was observed among these 23 proteins in cluster 1 (see Fig. 2, left). In cluster 2, a total of 19 proteins were identified, further divided into 3 sub-clusters, sub-cluster 1 included proteins FGF, HGF, and TWEAK with strong correlations, $r = 0.55$–0.75, $P < 2.6 \times 10^{-5}$ (see Fig. 2, left), subcluster 2 showed strong significant correlations between IL-6 with MCP-1 GCSF, and GROα with $r = 0.65$–0.87, $P < 1.94 \times 10^{-7}$). The third subcluster contained CD31, LIF, Eotaxin, TSLP, CD40L, IL-17A, IL-7, and MIP-1α with $r = 0.54$–0.82, $P < 1.92 \times 10^{-5}$ (see Fig. 2, left). In the post-cataract surgery group, cluster 1 (23 proteins) the r values ranged from 0.291 to 0.866, $P < 0.05$, whereas in cluster 2, the r values among 19 proteins range from 0.099 to 0.897. In the post-cataract

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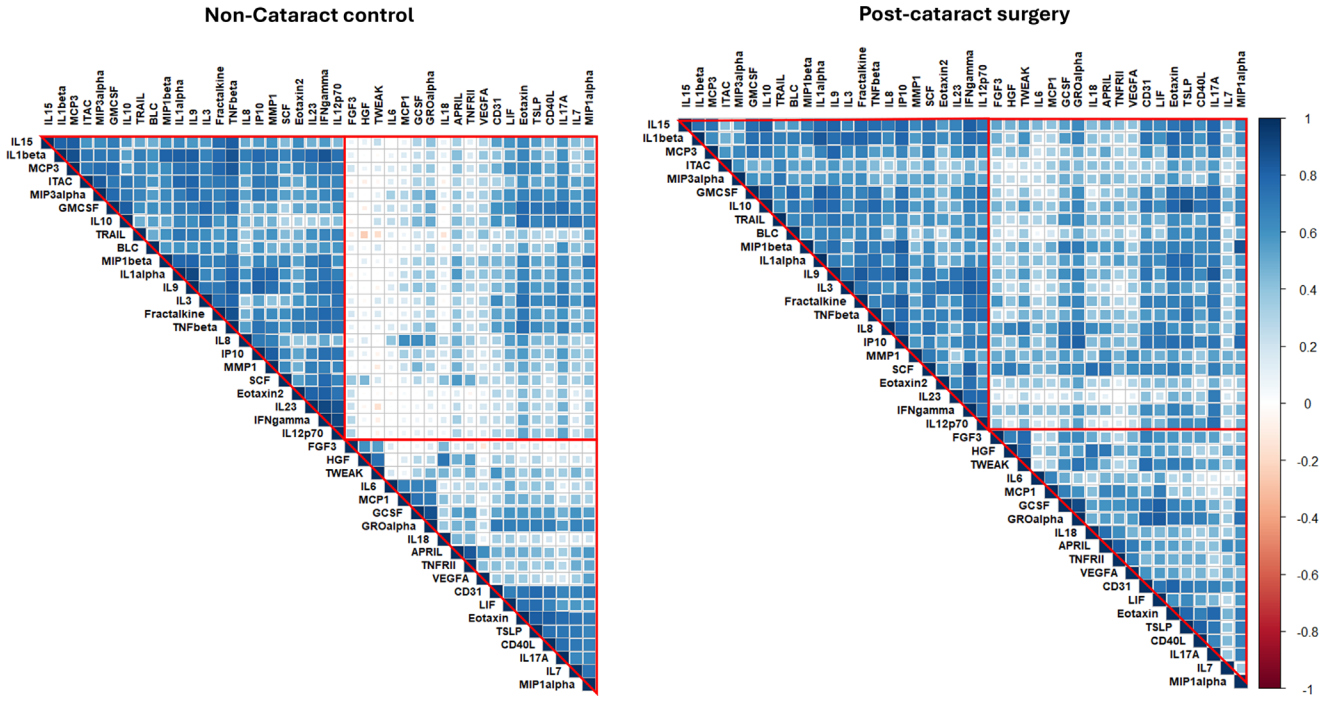

Figure 2. Pairwise correlations. Pairwise correlations between individual proteins were determined using the Pearson correlation. Left, non-cataract controls; Right, post-cataract surgery.

Table 3. Outcome of Stepwise Multivariate Logistic Regression

	Model 1		Model 2		Model 3	
Proteins	**OR 1***	***P* Value 1**	**OR 2***	***P* Value 2**	**OR 3***	***P* Value 3**
IFN-γ	0.61 (0.38–0.92)	0.0250	0.58 (0.34–0.94)	0.0330	0.57 (0.34–0.93)	0.0310
IL-7	2.03 (1.29–3.37)	0.0037	2.16 (1.22–4.11)	0.0120	2.16 (1.22–4.12)	0.0120
IL12p70	0.52 (0.32–0.8)	0.0040	0.55 (0.32–0.9)	0.0220	0.54 (0.31–0.89)	0.0210
IL-23	0.58 (0.36–0.89)	0.0160	0.55 (0.31–0.92)	0.0280	0.55 (0.31–0.92)	0.0290
LIF	1.91 (1.21–3.3)	0.0100	2.56 (1.51–4.91)	0.0016	2.57 (1.51–4.95)	0.0016
SCF	0.75 (0.49–1.13)	0.1800	0.58 (0.33–0.95)	0.0390	0.57 (0.33–0.94)	0.0370
TRAIL	0.66 (0.42–1)	0.0570	0.58 (0.34–0.94)	0.0310	0.58 (0.35–0.94)	0.0310

* Log2 transformed concentration (original unit = pg/mL).

surgery group, cluster 2 contained strong and significant correlations suggesting that these proteins are involved in post-cataract surgery AH's inflammatory environment (see Fig. 2, right).

We then assessed the differences in protein levels between the non-cataract and post-cataract surgery group, after adjusting for sex and age in a stepwise multivariate logistic regression (Table 3). The OR (95% confidence interval [CI]) for the per SD increase in the protein concentration for all 34 proteins is presented in Supplementary Table S3 and Table 3. Of these 34 proteins APRIL, MIP-1α, and VEGF were only significant in protein-only model (model 1). Seven proteins viz., IFNγ, IL-7, IL12p70, IL-23, LIF, SCF, and TRAIL (see Table 3) showed significant differences for protein only (model 1), protein + age (model 2), and protein + age + sex (model 3; see Table 3).

In our next analysis, the concentration values for each of the proteins were divided into 3 tertile groups containing 33% of the subjects, using the cutoff values from the post-cataract surgery group. For all comparisons, the first tertile was used as a reference to calculate OR for tertiles 2 and 3 (Supplementary Table S4, Table 4). In our analysis, we observed a significant association with increased AH levels of IL-1β, IL-7, IL12p70, LIF, and TRAIL. The OR for IL-7 (3.29, P = 0.480) and LIF (12.98, P = 0.0420) increased with higher concentrations, where a decrease in OR values was observed for IL-1b (0.23, P = 0.0420),

Table 4. Three Tertile Groups-Based Analysis

Protein	Tertile 2 OR*	Tertile 3 OR*	Adjusted *P* Trend
IL-1β	0.63 (0.19–2.04)	0.23 (0.08–0.66)	0.0420
IL-7	1.85 (0.69–4.96)	3.29 (1.15–9.42)	0.0480
IL12p70	0.88 (0.28–2.76)	0.3 (0.11–0.82)	0.0420
LIF	0.88 (0.36–2.17)	12.98 (2.16–77.85)	0.0420
TRAIL	0.83 (0.28–2.5)	0.31 (0.11–0.84)	0.0420
PCSII	16.7 (3.9–1.51)	27.83 (4.73–163.69)	9.1×10^{-10}

PCSII, post-cataract surgery inflammation index.
* Log2 transformed concentration (original unit = pg/mL).

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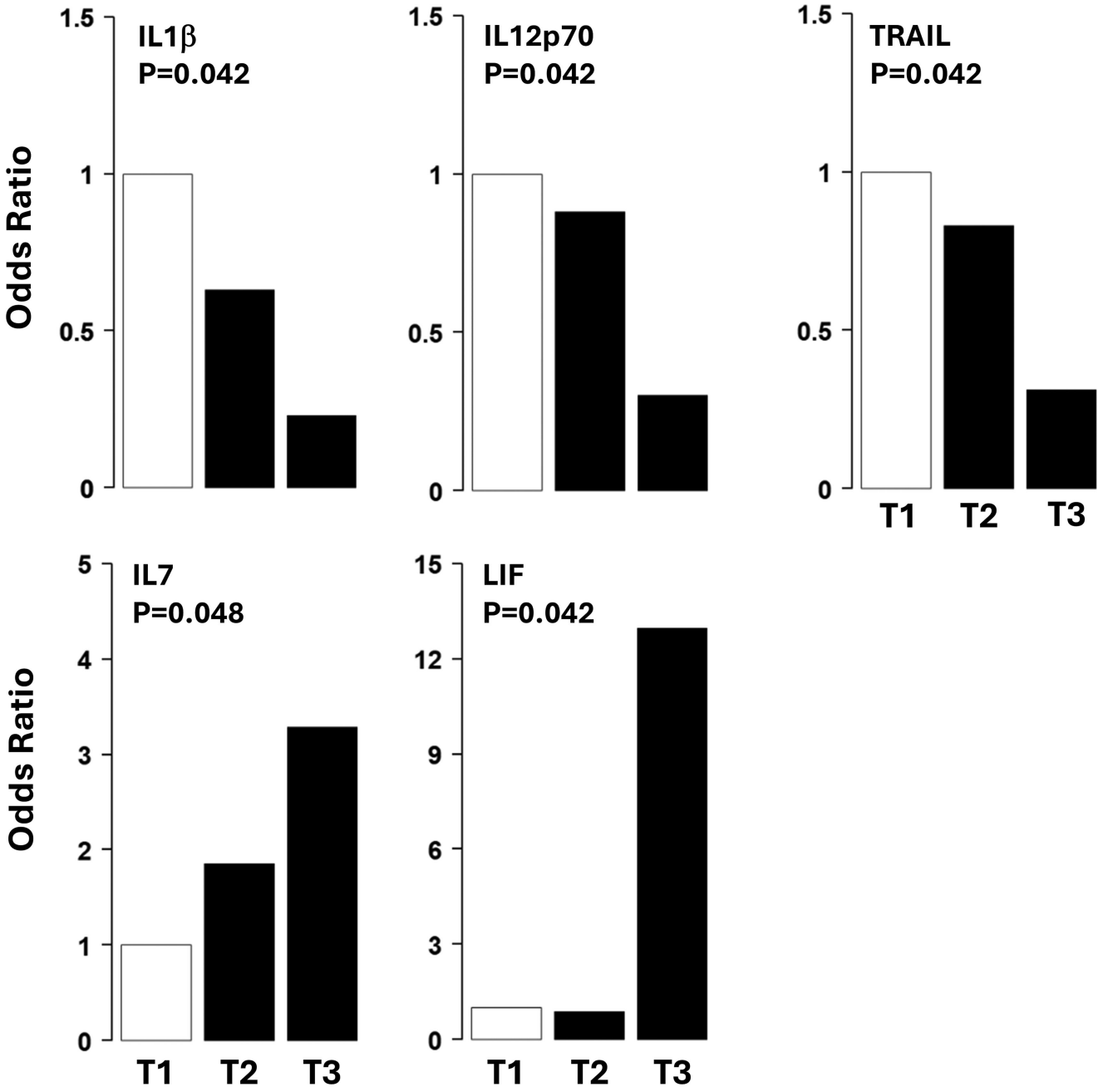

Figure 3. Odds ratio of three tertiles. AH molecule levels were divided into 3 tertiles each containing 33% of the samples. (**A**) IL-1β; (**B**) IL12p70; (**C**) TRAIL; (**D**) IL-7; and (**E**) LIF. Pearson's Chi-squared test with Yates' continuity correction was used to calculate the ORs. The chi-squared test for trend in proportions was used to calculate the P value of an overall trend. Only $P < 0.05$ is considered significant.

IL12p70 (0.3, $P = 0.0420$), and TRAIL (0.31, $P = 0.420$; Fig. 3, see Table 4). In this analysis, any increase in the concentration of IL-6 in tertiles 2 and 3 was associated with post-cataract surgery group (see Supplementary Table S4).

There Are Distinct Changes of Cytokines in AH After Cataract Surgery Compared to Non-Surgery Controls

We evaluated the potential association of each of these 34 molecules with cataract surgery. This was done by combining the concentration data for 34 proteins into a PCSII using ridge regression. The ridge regression coefficient values suggest that all 34 proteins' data contributed strongly to the PCSII (Fig. 4A). Boxplot analysis of the PCSII indicates a higher score value in the post-cataract surgery group than in the non-cataract group (Fig. 4B). The difference between the PCSII in the two groups was stronger than that of the individual molecules. On further analysis by dividing this score into 3 tertiles, the PCSII gave a higher OR of 27.83 ($P = 9.1 \times 10^{-10}$; see Table 4, Fig. 4C), for the subjects in the third tertile. The OR for post-cataract surgery donors in the second tertile was

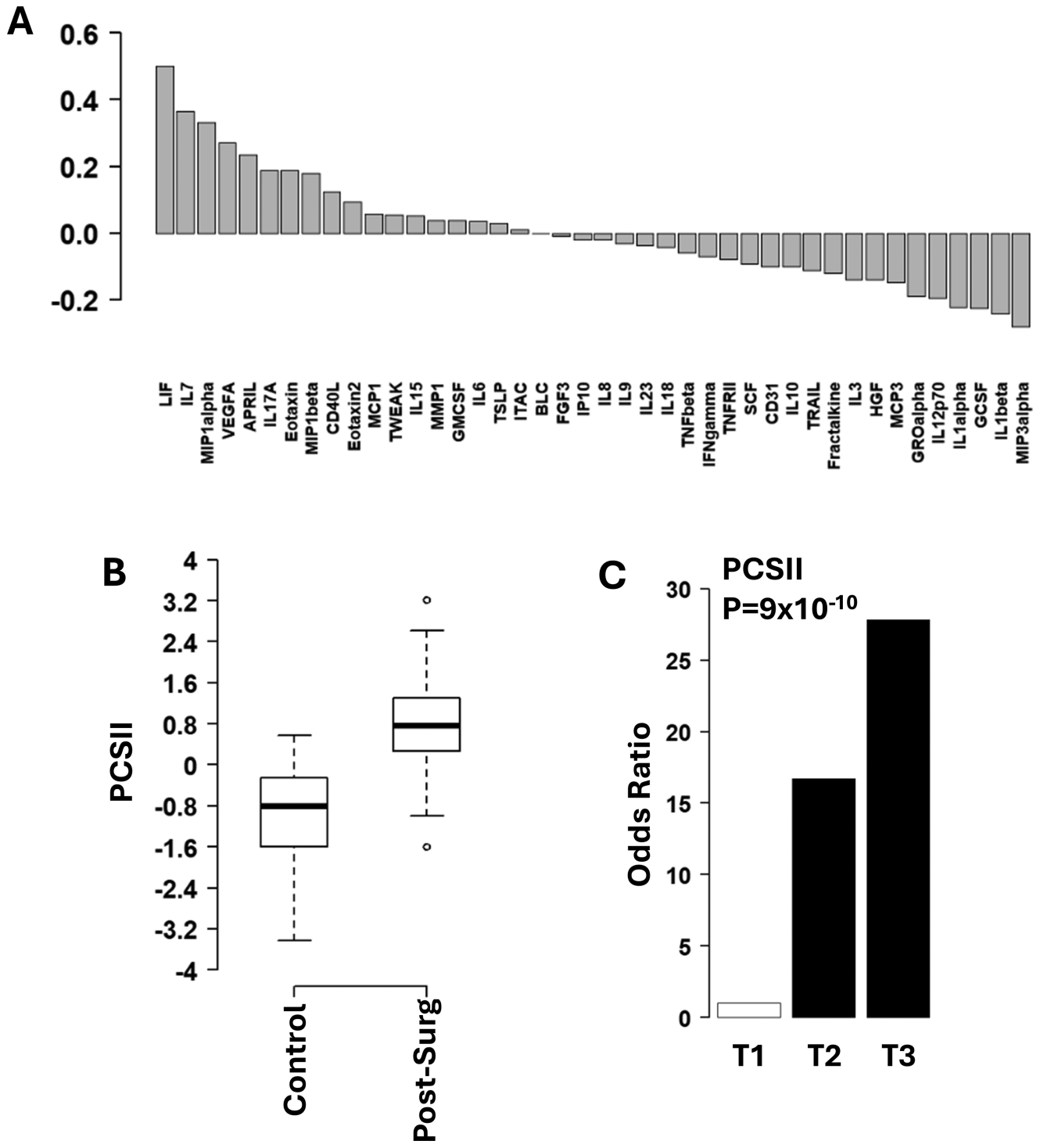

Figure 4. Ridge Regression Analysis. Thirty-four molecules were included in the ridge regression model to generate a post-cataract surgery inflammation index (PCSII). This score was then used to calculate the odds ratio (OR) for cataract surgery, comparing the upper two tertiles to the first tertile as a reference. (**A**) Relative contributions of the 34 proteins to the PCSII, calculated using ridge regression. (**B**) Boxplot showing distribution of the PCSII in healthy and post-cataract surgery donors. (**C**) Odds ratios for post-cataract surgery based on the PCSII, stratified into three tertiles.

16.7, suggesting that any elevation in concentrations of the proteins is associated with cataract surgery. Our PCSII score is found to be much better in terms of risk categorization, than individual molecules. The tertile-based results of the PCSII closely mirrored those observed for individual molecules such as IL-6, IL-7, MIP-1α, IL-17A, TNFRII, TSLP, and VEGFA. These findings indicate a similar trend, where an increase in AH concentration beyond the first tertile is associated with post-cataract surgery (Supplementary Fig. S4).

Discussion

Whether cataract surgery triggers chronic inflammation over a prolonged post-surgical period remains unclear. In this study, we examined 63 cytokines and growth factors in the AH of donors who had undergone cataract surgery and compared them with non-cataract individuals. Our findings revealed that several inflammatory cytokines, including IL-7, CD40L, MIP-1α, and LIF, were significantly elevated in the cataract surgery group compared to the healthy individuals, even 3 to 12 years after surgery. For the first time, this study suggests that cataract surgery may induce sustained chronic inflammation during the post-surgical period. These findings raise important questions about the sources of these cytokines and their potential clinical impacts.

Why do Some Inflammatory Cytokines Maintain Relatively High Levels After Cataract Surgery?

Despite advancements in phacoemulsification techniques and small-incision surgeries, the procedure still disrupts the BAB. The disruption and immune cells infiltration are widely recognized as the source of acute and subacute waves of inflammatory cytokines.[19,30] Typically, the BAB is restored within a few weeks postoperatively, effectively blocking the flow of cytokines into the AH. However, patients with pre-existing conditions or diabetes may require significantly more time to restore BAB function.[31] For patients without pre-existing BAB issues or diabetes (this study), elevated cytokine levels and chronic inflammation through the BAB pathway are less likely to occur.

Phacoemulsification is a procedure that breaks cataract fibers into pieces before their removal. However, some cortical or nuclear lens fragments, known as retained lens fragments, may remain in the eye. Although relatively rare (< 1%), retained lens fragments in the ocular chamber can be immunogenic.[32,33] Immune responses can occur within hours, and the intensity of the response is proportional to the size of the retained lens fragment.[34] Studies suggest that nuclear lens fragments elicit a stronger immune response compared with cortical lens fragments.[35] In a clinical study involving 54 patients with retained lens fragments examined 4 weeks after cataract surgery, 87% showed significant intraocular inflammation.[36] Severe immunogenic responses caused by retained lens fragments can lead to uveitis, also referred to as lens-induced uveitis.[19,37] When retained lens fragments dislocate into the posterior chamber, pars plana vitrectomy is often required.[33] In the current study, donors did not have a history of treatment for retained lens fragments. However, this does not rule out the possibility of microscopic lens particles triggering a continuous immunogenic response without causing overt ocular issues requiring medical attention.

In a mouse cataract surgery model, Jiang et al.[38] observed a massive immune response initiated by LECs. Twenty-four hours after surgery, significant upregulation of genes, such as CXCL1, S100a9, CSF3, COX2, CCL2, LCN2, and HMOX1, was detected in the lens epithelium compared with the controls. This study strongly suggests that, similar to other epithelial tissues, LECs can modulate immune responses, likely serving as a key mechanism in chronic inflammation after cataract surgery. However, additional studies are required to determine whether human lens epithelial cells express certain cytokines and receptors, and if their expression is upregulated following cataract surgery.

Although the lens has traditionally been considered an immune-privileged tissue, recent studies have revealed the presence of resident immune cells within the lens.[39] These immune cells are believed to play a crucial role in maintaining tissue homeostasis under normal physiological conditions.[40] However, they may become activated in response to tissue injury or stress. Notably, studies utilizing ex vivo chicken cataract surgery models have demonstrated that resident immune cells can become activated as early as 15 minutes post-injury.[41] This rapid immune response highlights the lens' capacity to initiate local immune surveillance, despite its immune-privileged status. In relation to the present study, key questions remain unanswered. It is currently unknown whether these resident immune cells persist within the lens capsule long after cataract surgery and, if so, whether they remain active for years post-surgery. Furthermore, it is unclear whether these cells contribute to the sustained production of the cytokines identified in the current research. Addressing these questions is critical to understanding the long-term immunological dynamics of the lens following surgical intervention.

What Are the Potential Impacts of These Cytokines?

Despite the eye being protected by both the blood-retinal barrier (BRB) and the BAB, growing evidence suggests that immunological factors and inflammation play a crucial role in various ocular disorders, including uveitis, diabetic retinopathy, dry eye disease, age-related macular degeneration (AMD), keratitis, myopia, and thyroid eye disease.[42,43] In the present study, we observed that CD40L, IL-6, IL-7, LIF, MIP-1α, and MCP-1 were either significantly increased or showed a trajectory elevation in the AH of donors with a history of cataract surgery compared with non-surgery controls.

These cytokines have been implicated in a range of ocular diseases. For instance, Yamakawa et al.[44] conducted a survey of AH cytokines in individuals with simple DR and compared them with controls without DR. Their study revealed significantly higher concentrations of CD40L, IL-6, and MIP-1α, along with 7 other cytokines, in the AH of patients with simple DR compared with control eyes. Elevated levels of TNF-α and IL-7 have been linked to poorer best-corrected visual acuity (BCVA) in patients with diabetic macular edema (DME).[45] Additionally, significantly increased levels of IL-7 have been detected in the tear fluid of patients suffering from contact lens-related dry eye.[46] Importantly, IL-7 has been shown to induce the production of both MCP-1 and IL-8 in retinal pigment epithelial (RPE) cells,[47] further implicating its role in ocular inflammation. Levels of IL-6, LIF, and VEGF-A, along with other cytokines, are substantially higher in patients with DR than in non-diabetic controls.[48] Moreover, LIF has been demonstrated to induce the production of key pro-inflammatory cytokines, such as IL-6 and TNF-α,[49] further highlighting its role in ocular inflammation.

The present study raises a critical question: Do elevated or decreased cytokine levels influence the pathogenesis and progression of ocular diseases, as well as LEC proliferation and differentiation—key mechanisms underlying PCO? For instance, elevated cytokine levels in the AH may directly impact anterior ocular tissues, such as the corneal endothelium and trabecular meshwork. Post-cataract surgery has been associated with an increased risk of corneal edema,

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which can progress to corneal decompensation requiring endothelial keratoplasty.[50] Inflammation is considered a key factor in this process. Inoue et al.[51] proposed that elevated AH MCP-1 levels might contribute to poor surgical outcomes in trabeculectomy for primary open-angle glaucoma (POAG). Consistently, our study detected a trajectory of MCP-1 elevation in the AH of donors with a prolonged history of cataract surgery, suggesting that post-cataract surgery inflammation may significantly influence other ocular functions.

Fibrosis is recognized as a critical mechanism in the initiation and progression of PCO.[52] A prominent example of this process is the TGF-β-mediated epithelial-mesenchymal transition (EMT), a well-established signaling pathway that promotes lens epithelial fibrosis.[53] This fibrotic process drives the transformation of lens epithelial cells into myofibroblast-like cells, which acquire enhanced cell mobility and adopt a more invasive phenotype. Although inflammation can play a protective role when tightly regulated, it often leads to adverse consequences when uncontrolled, resulting in tissue fibrosis and the development of chronic diseases.[54] The role of inflammation as a major driver of pathological fibrosis has been extensively documented across various organs, including the eyes.[55] Persistent inflammation is strongly linked to fibrotic tissue remodeling, where the immune response plays a central role in disease progression. In the eyes, immune responses are intricately involved in several fibrotic conditions. Corneal and conjunctival epithelial wound healing can lead to fibrotic scarring, whereas trabecular meshwork fibrosis is a significant contributor to the development of glaucoma. Additionally, lens fibrosis following cataract surgery is a primary cause of PCO, and retinal fibrosis is commonly observed in various degenerative eye diseases. Although the majority of research has focused on TGF-β-induced lens epithelial fibrosis, the specific roles of other inflammatory mediators remain less understood. Molecules, such as MCP-1, IL-6, IL-7, CD40L, MIP-1α, and LIF, may also play significant roles in the fibrotic process within the lens epithelium. Further investigation into these mediators is necessary to fully elucidate their contributions and to identify potential therapeutic targets for preventing or mitigating PCO and other fibrotic eye conditions.

We also observed several cytokines that were either significantly decreased or showed a trajectory of decline in the AH of donors with a long-term history of cataract compared with healthy controls. These changes may reflect abnormal immune function. For example, low levels of IFN-γ have been shown to impair tissue ability to combat intracellular pathogens. Conversely, suppressed IFN-γ levels are often associated with chronic inflammatory or autoimmune diseases, such as lupus or sarcoidosis. Additionally, low IFN-γ levels have been implicated in contributing to tissue fibrosis. Further studies are warranted to elucidate the precise roles of these cytokines in ocular health and disease after cataract surgery.

The present study has several limitations. First, the smaller sample size is a key limitation, as it may reduce the statistical power of the analysis. Second, detailed clinical and phenotypical information, such as visual acuity and the degree of posterior capsule opacification prior to requiring laser surgery, was unavailable. Finally, the AH samples were collected postmortem, and variability in sample collection and immediate processing among individual operators may have influenced the results. The strength of our study includes measurement of multiple proteins ($n = 63$) in a multiplex format, which has a higher replicability and application in clinics. The proteins included belongs to three major categories of activation of inflammation (sTNFRII, GMCSF, IL-1β, IL-6, and IL-12p70), and cell and vascular growth factors (FGF, HGF, GCSF, and VEGF). A key highlight of our study is the use of a machine learning approach to transform protein concentration data into an individualized score. This score effectively differentiates AH samples from healthy individuals and post-cataract surgery donors, categorizing the latter into risk groups even 3 to 12 years after surgery.

In conclusion, our study surveyed a range of cytokines and growth factors, revealing that cataract surgery may induce a chronic inflammatory environment in the AH, persisting even during extended post-operative periods.

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