

Preoperative profile of inflammatory factors in aqueous humor correlates with postoperative inflammatory response in patients with congenital cataract

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Purpose: To measure the aqueous humor concentrations of inflammatory factors in patients with congenital cataract and to investigate the relationship between the levels and postoperative inflammatory responses.

Methods: Aqueous humor samples were prospectively collected from 65 eyes of children with congenital cataracts from January to June 2015. The levels of 41 inflammation-related cytokines, chemokines, and growth factors in aqueous humor were measured using multiplex bead immunoassay. Data on patient demographics and postoperative inflammatory response evaluation of posterior capsule opacification (EPCO) scores were collected for correlation analysis of short- and long-term postoperative inflammatory responses, respectively.

Results: Fifteen inflammatory factors were differentially expressed between congenital cataract and age-related cataract. EGF and IL-3 were positively correlated, whereas IL-8 and MCP-1 were negatively correlated with age. TNF α , IL-17A, IL-3, and sCD40L were preferably expressed in specific morphological types of congenital cataract. One month and 3 months postoperatively, PDGF-AA exhibited a positive correlation with the EPCO scores, whereas IL-1RA exhibited a negative correlation. Macrophage-derived chemokine (MDC) showed a positive correlation with the EPCO scores 1 year postoperatively.

Conclusions: This study provided a comprehensive preoperative profile of inflammatory factors and their correlations with postoperative inflammatory responses in patients with congenital cataract. These factors may serve as potential biomarkers to predict the postoperative inflammatory response. These findings will also facilitate the development of anti-inflammatory medications in the perioperative period.

Congenital cataract, an opacity of the lens at birth or at an early stage of childhood [1], is the leading cause of avoidable childhood blindness worldwide [2]. Cataract surgery with intraocular lens (IOL) implantation is the most common and effective treatment for cataract [3]. However, congenital cataract surgery carries a high risk of developing postoperative complications due to uncontrolled inflammatory responses [4-7]. Over the short term, prolonged and serious postoperative inflammation can be observed on the ocular surface and in the anterior chamber [5]. Over the long term, posterior capsular opacification (PCO; [7]), secondary glaucoma [6], and fibrinous uveitis [4] may occur.

Previous studies indicated that TGF- β 2 is positively correlated with age in cataract eyes [8]. In addition, the

levels of other proinflammatory cytokines, such as IL-1, IL-2, IL-4, IL-18, and IFN- γ , are considerably higher in children than those in adults [9]. Although the dysregulation of cytokines and inflammatory factors, such as TGF- β and IL-1, is a known contributor to postoperative inflammatory lens fibrosis [10], the preoperative profile of these factors in the aqueous humor and their relationship with the postoperative inflammatory response in patients with congenital cataract is not well characterized.

Our group previously identified that the increased IL-6 level may contribute to inflammation-related cataractogenesis [11]. A recent pilot study further revealed several cytokines were dysregulated in congenital cataract, and their levels were correlated with the development of PCO [12]. However, the dynamic changes in the inflammatory profile with age and the relationship between inflammatory factors and cataract morphology have not been assessed. In addition, there has been no quantitative evaluation of the postoperative inflammatory responses. In this study, we aimed to provide a

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comprehensive preoperative profile of inflammatory factors in congenital cataract at different ages and in distinct cataract morphological types. Additionally, we investigated the correlation of these factors with postoperative inflammatory responses by measuring the evaluation of posterior capsule opacification (EPCO) scores.

METHODS

Subjects and enrolment criteria: Patients with congenital cataract undergoing cataract extraction with or without IOL implantation were prospectively recruited during preoperation screening at the Zhongshan Ophthalmic Center (ZOC), Guangzhou, China, from January to June 2015. This study followed the tenets of the Declaration of Helsinki, adhered to the ARVO statement on human subjects and was approved by the Ethical Review Committee of Zhongshan Ophthalmic Center. Informed written consent was obtained from at least one guardian of each participating child.

A patient was considered eligible upon meeting the following inclusion criteria [1]: diagnosed with congenital cataract caused by hereditary or developmental disorders based on the judgment of qualified pediatricians, ophthalmologists, or medical records according to the International Classification of Disease and Codes (ICD-11) [2], age 0–10 years old [3], and gestational age at birth 37–42 weeks. The exclusion criteria included the presence of any of the following [1]: age >10 years [2]; gestational age at birth <37 weeks (premature) or >42 weeks (post-mature) [3]; a recent history of ocular infection or tropical/systemic immunosuppressive medications within 1 month [4]; the presence of any ocular or systemic comorbidities, including but not limited to a history of glaucoma, ocular trauma, corneal disorders, persistent hyperplastic primary vitreous, rubella, Lowe syndrome, capsular fibrosis; and [5] inability to participate in the post-surgery follow-up within at least 3 months.

Surgical approaches and postoperative regimen: Demographic information, including gender, age, laterality, and morphology, was obtained from each eligible patient. For bilateral cases, the first operated eye was included in the analyses (Random number generator tools, Version 1.4, Duote Co., Wuhu, China). All patients underwent a thorough ophthalmic evaluation, including slit-lamp examination, fundus photography, and B-scan ultrasonography. All patients underwent cataract surgery performed by an experienced pediatric cataract surgeon (Yizhi Liu) using a standardized technique previously described [13]. Standardized lens aspiration with IOL implantation, posterior continuous curvilinear capsulorhexis, and anterior vitrectomy [I/A+IOL+PCCC+A-Vit])

was performed. Briefly, after conjunctival peritomy, a superior scleral tunnel incision was created using a 3.2-mm keratome. An anterior capsulotomy of 4.0–5.0 mm was made in a continuous curvilinear fashion [13]. The nucleus and cortex were removed using a manual irrigation/aspiration device or an automated vitrectomy instrument. The vitrectomy instrument was used to create a central posterior capsulotomy and to perform a limited anterior vitrectomy. All IOLs used were exclusively the three-piece AcrySof IQ IOL (Alcon Laboratory, Fort Worth, TX) and achieved in-the-bag implantation. All surgeries were performed under general anesthesia. Postoperatively, TobraDex eye drops (0.3% tobramycin and 0.1% dexamethasone; Alcon, Aliso Viejo, CA) were used 6 times per day, and TobraDex eye ointment (0.3% tobramycin, and 0.1% dexamethasone; Alcon) was applied once per night for 2 weeks. From 2 weeks to 1 month postoperatively, the eye drops were used 4 times per day. For the second postoperative month, the patient switched to pranoprofen eye drops 4 times per day (Senju Pharmaceutical Co. Ltd., Osaka, Japan).

Aqueous humor collection: Aqueous humor samples (100–200 μ l) were collected at the beginning of the operation. A 30-gauge needle on a tuberculin syringe was used to aspirate aqueous humor from the anterior chamber via a limbal paracentesis before the scleral tunnel incision. All samples were obtained before any conjunctival or intraocular manipulation to avoid breakdown of the blood–aqueous barrier associated with surgical trauma. The aqueous humor samples were stored immediately under -80°C until analysis.

Measurement of inflammatory factors: The concentrations of inflammatory factors were analyzed using a multiplex bead immunoassay system (Milliplex Human Cytokine Kit; Millipore Corp., Billerica, MA). The assay was performed according to the manufacturer's instructions and analyzed using the Bio-Plex Suspension Array System (Bio-Plex200; BioRad, Hercules, CA) [14]. A 25 μ l volume of aqueous humor sample was used for the measurement of multiple inflammatory factors. The detection range was 1 pg/ml to 10,000 pg/ml. Each sample was measured in duplicate. The inflammatory factors were analyzed simultaneously and are listed in Table 1.

Assessment of postoperative inflammatory response and EPCO scores: For patients who underwent the I/A+IOL+PCCC+A-Vit surgical procedure, the postoperative inflammatory response was evaluated with EPCO scores at 1 month, 3 months, and 1 year after surgery. The EPCO scores were evaluated after the slit-lamp examination [15]. Pupils were dilated with 1% tropicamide and 2.5% phenylephrine. Digital retroillumination photographs were taken with a biomicroscope using a digital photo adaptor (BX 900H

TABLE 1. THE INFLAMMATORY FACTORS MEASURED BY MULTIPLEX ASSAY KIT.

| Cytokines | Interleukin (IL)-1 α |
|----------------|--|
| | IL-1 β |
| | IL-1RA |
| | IL-2 |
| | IL-3 |
| | IL-4 |
| | IL-5 |
| | IL-6 |
| | IL-7 |
| | IL-9 |
| | IL-10 |
| | IL-12p40 |
| | IL-12p70 |
| | IL-13 |
| | IL-15 |
| | IL-17A |
| | Tumor necrosis factor (TNF)- α |
| | TNF β / Lymphotoxin alpha (LTA) |
| | Interferon- γ (IFN- γ) |
| | IFN- α 2 |
| | Soluble CD40 ligand (sCD40L) |
| Chemokines | Granulocyte colony-stimulating factor (G-CSF) |
| | Granulocyte-macrophage colony-stimulating factor (GM-CSF) |
| | Monocyte chemotactic protein (MCP)-1/CCL2 |
| | MCP-3 |
| | Macrophage inflammatory protein-1 α (MIP-1 α)/CCL3 |
| | MIP-1 β /CCL4 |
| | IL-8/CXCL8 |
| | Interferon-inducible protein-10 (IP-10)/CXCL10 |
| | Fractalkine |
| | Macrophage-derived chemokine (MDC) |
| | Eotaxin (CCL11) |
| | Growth regulated oncogene (GRO α)/CXCL1 |
| | Regulated upon activation, normal T cell expressed and presumably secreted (RANTES)/CCL5 |
| Growth factors | Epidermal growth factor (EGF) |
| | Fibroblast growth factor (FGF-2) |
| | TGF- α |
| | Platelet-derived growth factor (PDGF)-AA |
| | Platelet-derived growth factor (PDGF)-AB |
| | Flt-3L |
| | Vascular endothelial growth factor (VEGF) |

TABLE 2. EPCO GRADING SCALE.

| Grade | Description |
|-------|---|
| 0 | No visible PCO |
| 1 | Minimum wrinkling of the capsule with a fine layer of LECs |
| 2 | Mild honeycomb PCO; thicker layer of LECs with dense fibrosis |
| 3 | Classic Elschnig pearls; very thick layer of LECs |
| 4 | Severe opacity with a darkening effect |

LECs=lens epithelial cells; PCO=posterior capsule opacification

Photo Slit Lamp, Haag-Streit AG, Bern, Switzerland) and a high-resolution digital camera. The EPCO system [16] was used to assess PCO (EPCO2000, developed by Tetz MR and Associates, Berlin, Germany). In this system, the amount of opacification within the pupil area is graded from 0 to 4 (Table 2; [15]). The total PCO score was calculated by multiplying the opacification density score by the percentage of the opacified capsule area (Appendix 1).

Statistical analysis: The data were processed and analyzed using SPSS (version 13.0; SPSS, Chicago, IL). The Shapiro-Wilk test was used to evaluate the normality of distribution for all variables. For the variables fitting the normal distribution, data were recorded as the mean \pm standard deviation (SD). The two-tailed independent samples *t* test was used to compare the means between two groups. One-way ANOVA followed by Bonferroni's post-hoc comparison test was performed to compare the means among more than three groups. For the variables not fitting the normal distribution, data were recorded as the median and the 25th to 75th interquartile range. The Mann-Whitney U test or the Kruskal-Wallis test was used to test the intergroup differences. Correlations between the concentrations of inflammatory factors and postoperative inflammatory responses or age were calculated with partial correlation (Spearman) analysis. For each significantly correlated inflammatory factor as a dependent variable, any other correlated factors were enrolled in the least absolute shrinkage and selection operator model (LASSO model) [17] to screen for the multicollinearity and prescreen for the true influencing factors. The remaining correlated variables after filtering with the LASSO model were further analyzed with multivariate linear regression to evaluate the true effect of each independent predictor on the dependent variable. A *p* value of less than 0.05 was considered statistically significant for the tests.

RESULTS

Demographic and clinical characteristics of patients: A total of 65 eyes (subjects) fulfilled the inclusion criteria and were enrolled in the study. The demographic and clinical data of the included subjects are summarized in Table 3. There was a preponderance of male (56.9%) and bilateral (72.3%) patients. Nuclear (29.2%) and total (27.7%) cataracts were the most common morphological types, accounting for more than half of all cataracts. I/A+IOL+PCCC+A-Vit was performed in 83.1% of all subjects. The median age of the patients was 4 years old, with an interquartile range of 2 to 5 years old. Patients who underwent the single surgical procedure I/A+IOL+PCCC+A-Vit were included in the correlation analysis between the inflammatory factors and the postoperative inflammatory response, considering the potential influence of different surgical procedures. The other 11 samples were used only for the inflammatory factors analysis.

Inflammatory factors profile characteristics: The 41 inflammatory factors examined in the panel were summarized in Appendix 2. The VEGF level was beyond the upper limit (10,000 pg/ml) in ten subjects.

Inflammatory factor profiles change dynamically with age: EGF ($\rho=0.281$, $p=0.024$) and IL-3 ($\rho=0.294$, $p=0.017$) were positively correlated with age. IL-8 ($\rho=-0.460$, $p<0.001$) and MCP-1 ($\rho=-0.494$, $p<0.001$) were negatively correlated with age (Figure 1).

Inflammatory factors profile between different cataract morphological types: Interestingly, several inflammatory factors, including TNF α , IL-17A, IL-3, and sCD40L, are differentially expressed between different morphological types. The lamellar cataract group exhibited statistically significantly higher concentrations of TNF α ($p=0.001$) and IL-3 ($p=0.010$) than the nuclear cataract group, as well as statistically significantly higher levels of IL-17A ($p=0.012$) and IL-3 ($p=0.011$) than the total cataract group. The total cataract group exhibited a statistically significantly higher level of sCD40L ($p=0.004$) than the nuclear cataract group (Figure 2).

TABLE 3. PATIENT BASELINE CHARACTERISTICS.

| Characteristic | Median (25th, 75th interquartile range) |
|-------------------------------------|---|
| Age | 4.0 (2.0,5.0) |
| | Number (%) |
| Gender (n=65) | |
| Male | 37 (56.9) |
| Female | 28 (43.1) |
| Cataract Laterality (n=65) † | |
| Bilateral | 47 (72.3) |
| Unilateral | 18 (27.7) |
| Cataract Morphology (n=65) | |
| Nuclear | 19 (29.2) |
| Total | 18 (27.7) |
| Lamellar | 10 (15.4) |
| Posterior subcapsular | 9 (13.8) |
| Mixed or others | 9 (13.8) |
| Cataract Surgical Procedures (n=65) | |
| I/A+IOL+PCCC+A-Vit‡ | 54 (83.1) |
| I/A+PCCC+A-Vit | 4 (6.2) |
| I/A+IOL | 6 (9.2) |
| Missing data | 1 (1.5) |

†For bilateral cases, the first operated eye was included in the analyses. ‡Patients who underwent the single surgical procedure “I/A+IOL+PCCC+A-Vit” were included in the correlation analysis between the inflammatory factors and the postoperative inflammatory response, considering the potential influence of different surgical procedures. The other samples were only used for the inflammatory factors analysis. I/A+IOL+PCCC+A-Vit: lens aspiration with IOL implantation, posterior continuous curvilinear capsulorhexis and anterior vitrectomy; I/A+PCCC+A-Vit: lens aspiration with posterior continuous curvilinear capsulorhexis and anterior vitrectomy; I/A+IOL: lens aspiration with IOL implantation.

Post-surgery inflammatory responses between surgical procedure subgroups: Statistically significant higher EPCO scores were observed in the I/A+IOL+PCCC+A-Vit group compared with the I/A+PCCC+A-Vit group at 1 year after surgery (1.184 ± 0.354 versus 0.473 ± 0.425 , $p=0.010$), demonstrating the inflammatory responses induced by IOL implantations (Figure 3).

Inflammatory factors profile between gender and cataract laterality subgroups: No statistically significant differences in inflammatory factor levels were observed between different genders (Appendix 3). Only the VEGF level was statistically significantly higher in unilateral cataract than in bilateral cataract ($p=0.041$; Appendix 4).

Correlation between inflammatory factors and postoperative inflammatory responses: A summary of the post-surgery

inflammatory responses is shown in Appendix 5. According to the Spearman correlation analysis, PDGF-AA ($\rho=0.497$, $p=0.030$; $\rho=0.647$, $p=0.009$) was positively correlated, while IL-1RA ($\rho=-0.402$, $p=0.013$; $\rho=-0.413$, $p=0.029$) was negatively correlated with the EPCO scores at 1 and 3 months after surgery. TNF β ($\rho=-0.402$, $p=0.046$) and IL-3 ($\rho=-0.524$, $p=0.006$) were also negatively correlated with the EPCO scores at 3 months after surgery. After multivariate analysis (LASSO and multivariate linear regression), IL-1RA ($\beta=-0.382$, $p=0.015$; $\beta=-0.336$, $p=0.044$) and PDGF-AA ($\beta=0.415$, $p=0.044$; $\beta=0.564$, $p=0.006$) remained statistically significant at 1 and 3 months after surgery. IL-7 ($\rho=0.402$, $p=0.005$) and MDC ($\rho=0.326$, $p=0.036$) were positively correlated with EPCO scores in univariate analysis 1 year after surgery, and only MDC remained statistically significant in the multivariate analysis ($\beta=0.129$, $p=0.036$). Details are included in Table 4, Appendix 6, and Figure 4.

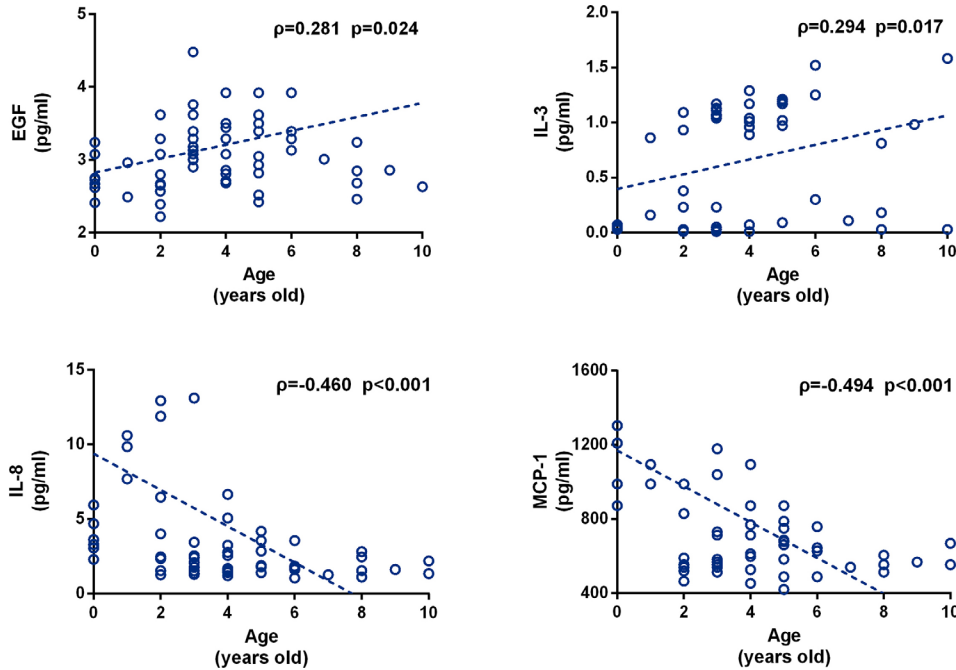


Figure 1. Correlation of inflammatory factors with age. The Spearman correlation test showed that the levels of EGF and IL-3 had statistically significant positive correlations with age, while the levels of IL-8 and MCP-1 had statistically significant negative correlations with age.

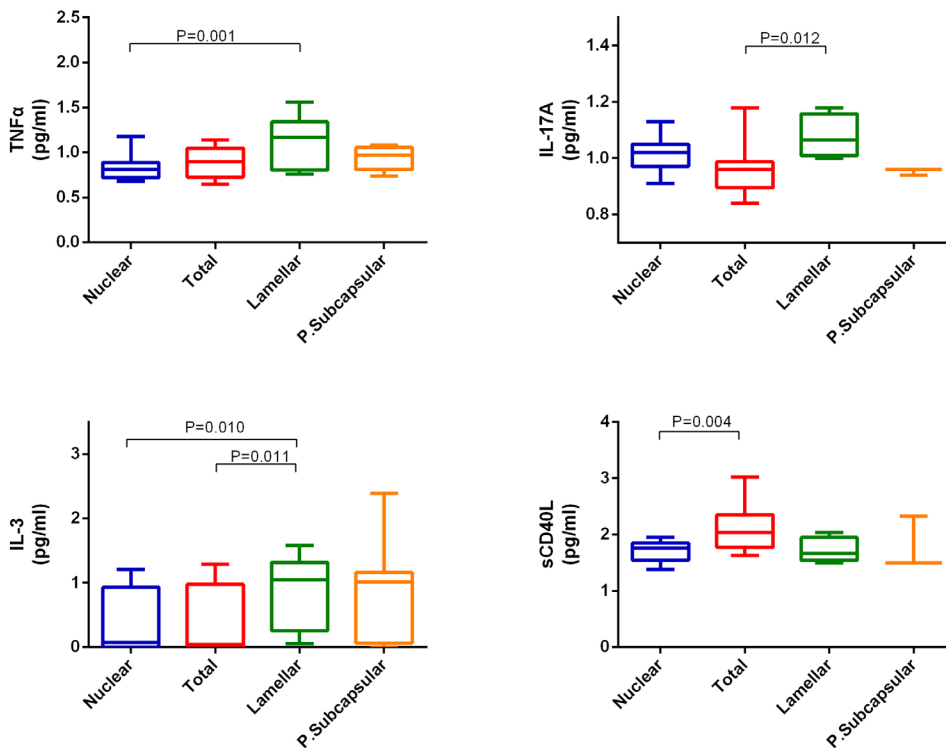


Figure 2. Box-whisker plots showing levels of inflammatory factor expression in different congenital cataract morphology subgroups. Solid horizontal lines indicate the median and the 25th–75th interquartile range level. A Kruskal–Wallis test with a post hoc Mann–Whitney test was performed between groups, and a statistically significant difference was accepted at $p<0.0125$ by Bonferroni correction.

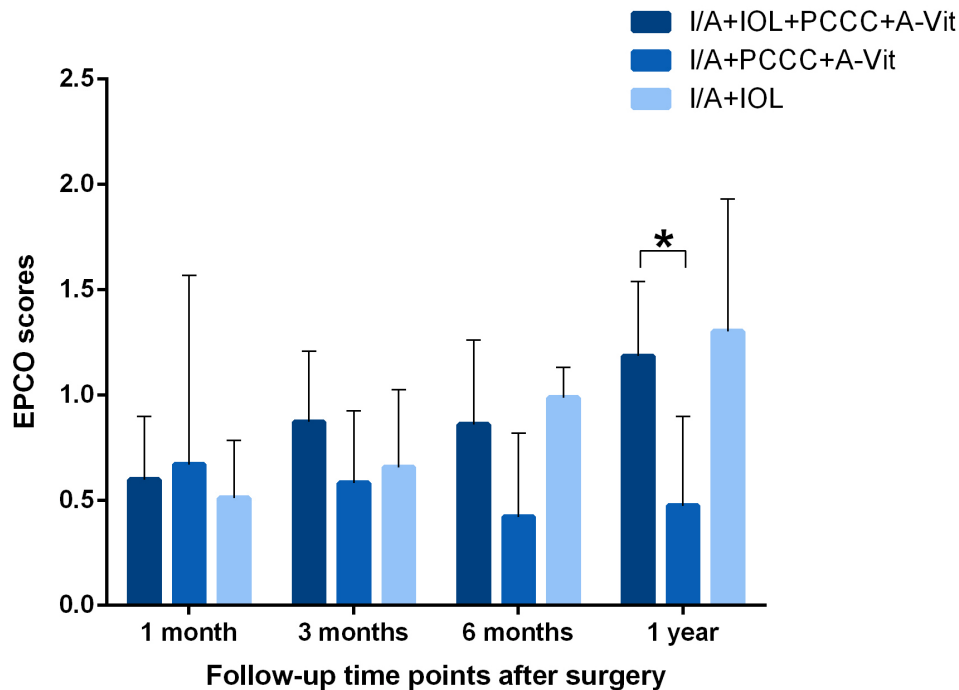


Figure 3. Histogram of EPCO scores in different surgical procedures groups. ANOVA with least significant difference (LSD) post-hoc comparisons were performed to compare between groups. “*” indicated a statistically significant difference with $p < 0.05$.

DISCUSSION

Based on the ZOC platform and using the world’s largest clinical database and biobank specifically for congenital cataract [18], we measured the inflammatory factor profiles in the aqueous humor of patients with congenital cataract and investigated the relationships between the levels and postoperative inflammatory responses. To the best of our knowledge, this is the first study to trace dynamic changes in intraocular inflammatory factor profiles during the first decades of life. We found that most cytokines, including interleukin, TNF, and IFN family members, remain constant with age, indicating a relatively stable state from birth to early childhood. In contrast, EGF, IL-3, IL-8, and MCP-1 were statistically significant correlated with age. Given that cytokines in the aqueous humor are primarily secreted from ciliary body capillaries and subsidiarily from uveal stromal immunocytes [19], the differential expression of inflammatory factors at different ages could be due to the development of uveal stromal immunocytes. Moreover, because lens epithelial cells secrete multiple inflammatory factors and influence the inflammatory microenvironment in aqueous humor, it is plausible that the factors detected changing with age may be concerned with the cataractous lenses, particularly as they progress [20,21].

The comparison of inflammatory factor profiles between different morphology subgroups revealed elevated levels of

several proinflammatory factors, including TNF α , IL-17A, and IL-3, in lamellar cataracts compared with the levels in other subgroups. This research expanded the findings of the previous study by Yamamoto et al., which demonstrated a considerably higher TGF- β_2 concentration in lamellar cataract than in nuclear and posterior subcapsular cataract [22]. Different morphological types of congenital cataract may have distinct genetic variations, age of onset, and progression characteristics [23]. Lamellar cataracts are typically acquired after birth, but the severity and area can progress with age in most cases [24]. The slow progression of lamellar congenital cataract indicates the potential influence of the local ocular microenvironment. Among these identified factors, TNF α promotes the production of the extracellular matrix (ECM), which is an important regulator of lens epithelial cells (LECs) proliferation and differentiation [25]. The role of the other factors during lamellar cataractogenesis requires further investigation.

Inflammatory factors could contribute to postoperative inflammatory responses via different mechanisms. The inflammatory factors could stimulate the proliferation and epithelial-mesenchymal transition (EMT) of LECs, which leads to the development of PCO [10]. Thus, we used the EPCO scores to evaluate the severity of the postoperative responses (1 month, 3 months, and 1 year after surgery). MDC, IL-7, and PDGF-AA are positively correlated with postoperative EPCO, whereas IL-1RA, TNF β , and IL-3

TABLE 4. THE UNIVARIATE AND MULTIVARIATE REGRESSION ANALYSIS OF THE INFLAMMATORY FACTORS WITH INFLAMMATORY RESPONSES AFTER SURGERY.

| Dependent variable | Univariate analysis (spearman correlation) | | | Multivariate analysis (LASSO and multivariate linear regression) | | | | |
|--|---|--------|--------------|---|--------|----------------------|--|--|
| | predictor | ρ | p (2-tailed) | Standardized coef- ficient (β) | p | Adjusted R Square | Significance of Regres- sion in ANOVA | |
| EPCO (one month after surgery, n=33) | IL-1RA | -0.402 | 0.013 | -0.382 | 0.015* | 0.161 | 0.025 | |
| | PDGF-AA | 0.497 | 0.03 | 0.415 | 0.044* | | | |
| EPCO (three months after surgery, n=30) | IL-1RA | -0.413 | 0.029 | -0.336 | 0.044* | 0.366 | 0.011 | |
| | IL-3 | -0.524 | 0.006 | -0.413 | | 0.682 | | |
| | TNF β | -0.402 | 0.046 | -0.215 | | 0.418 | | |
| | PDGF-AA | 0.647 | 0.009 | 0.564 | 0.006* | | | |
| EPCO (one year after surgery, n=22) | MDC | 0.326 | 0.036 | 0.129 | 0.036* | 0.147 | 0.036 | |
| | IL-7 | 0.402 | 0.005 | out [#] | / | | | |

*P value <0.05 indicating statistical significance. # The inflammatory factor was excluded after filtered by LASSO model and was not further analyzed by multivariate linear regression.

are negatively correlated. IL-1RA, PDGF-AA, and MDC remained statistically significant in multivariate linear regression. As reported by Jakobsson et al. [26], the MDC level is upregulated after irritation induced by intraocular surgical manipulation. MDC is a typical inflammatory chemokine activated in response to proinflammatory stimuli and participates in the inflammatory response by attracting immune cells positioned to the inflammatory reaction area [27]. IL-1RA is a well-known anti-inflammatory cytokine involved in the inflammatory PCO reaction via the IL-1 α and IL-1 β blockade [28]. We found that IL-1RA exhibited a statistically significant negative correlation with the EPCO scores 1 month and 3 months after surgery, suggesting IL-1RA has an anti-inflammatory function and may help to prevent PCO after surgery. Taken together, the identified factors that correlated with the postoperative EPCO scores can serve as potential biomarkers to predict postoperative inflammatory responses.

A study recently published by Sauer et al. [12] also examined the correlation between a series of 27 inflammatory factors with postoperative PCO at 3 months after surgery. A statistically significant correlation was found with IL-1 β , IL-1R α , IFN- γ , TNF α , IL-6, IL-10, IL-8, MCP-1, IP-10, G-CSF, and FGF. The different findings between their study and ours could be interpreted by considering the demographic differences and cataract status of the recruited patients, the conditions of the sample storage, the accuracy of the inflammatory factor measurement assay, and the PCO quantification methods. Further studies are still warranted to confirm the associations.

The principal strengths of this study included the following aspects. First, using a larger congenital cataract cohort than that in the previous study [12], we traced the dynamic change in the inflammatory profile with age and cataract morphology for the first time. Second, the inflammatory factor profile was detected using an advanced multiplex bead immunoassay system that allows for the comprehensive

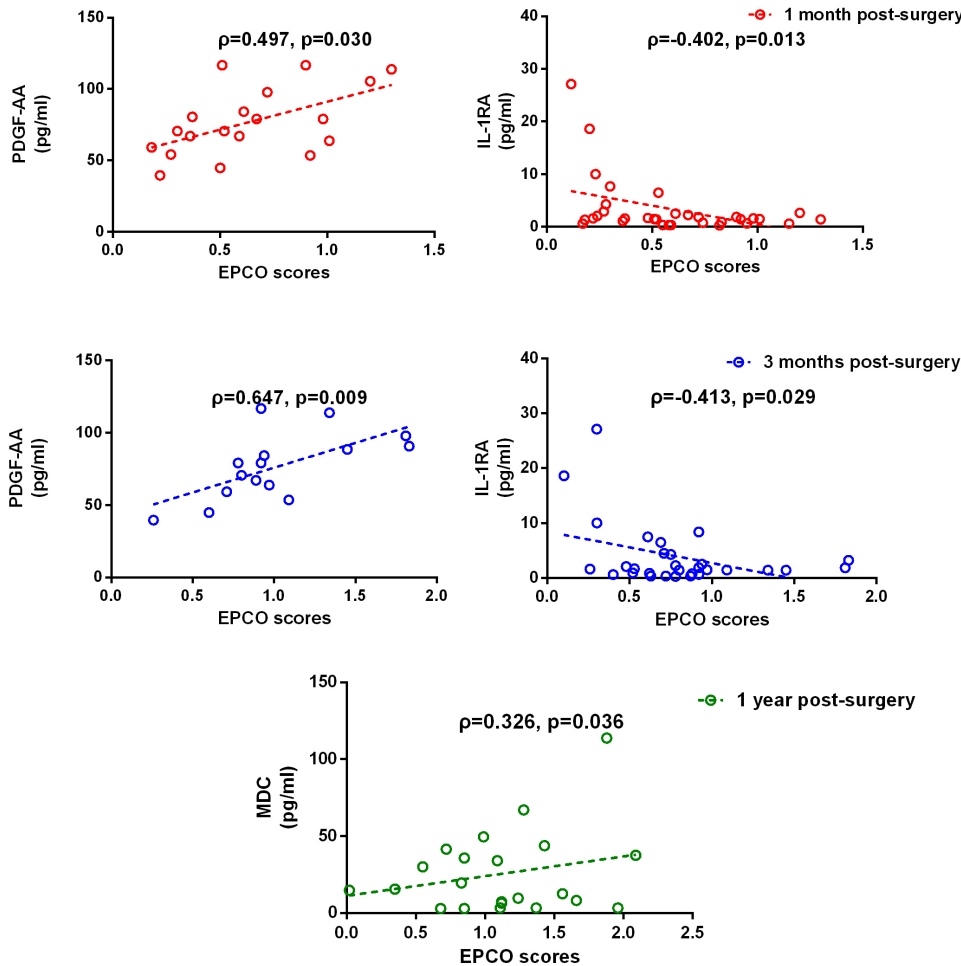


Figure 4. Correlation of inflammatory factors with post-surgery EPCO scores. The Spearman correlation test showed that PDGF-AA was positively correlated, while IL-1RA was negatively correlated with the evaluation of the posterior capsule opacification (EPCO) scores at 1 and 3 months after surgery. MDC was statistically significantly positively correlated with the EPCO scores.

measurement and analysis of the cytokine profiles with a small sample volume (typically 25 µl for each sample), which is barely sufficient for traditional enzyme-linked immunosorbent assay (ELISA). This technique has also better reproducibility and sensitivity than traditional ELISA [14]. However, potential limitations of the study should be mentioned. First, although we carefully controlled the perioperative medications of each subject and conducted partial correlations to minimize the potential confounding factors, the use of topical anti-inflammatory medications may still have influenced the aqueous immune milieu. Second, the changes in the inflammatory factors with age may have resulted from an over-representation of a certain cataract type in each age group. Further validation with a larger sample size is required to minimize this confounding factor. Third, healthy subjects with matched age would be theoretically ideal controls but ethically problematic to obtain. Postmortem ocular samples from an eye bank could be used as possible controls in the future.

In conclusion, we provided a comprehensive preoperative profile of inflammatory factors and their correlations with the postoperative inflammatory responses in patients with congenital cataract. These factors may serve as potential biomarkers to predict the postoperative inflammatory response and facilitate the development of anti-inflammatory medications in the perioperative period. Further studies are required to demystify the role of differentially expressed inflammatory factors in cataractogenesis of various morphologies, and to identify the underlying mechanism by which intraocular inflammatory factors induce postoperative inflammatory complications in congenital cataracts.

APPENDIX 1. EPCO SCORE ASSESSMENT.

To access the data, click or select the words “[Appendix 1.](#)”
A, Slit-lamp retroillumination photograph of a pseudophakic eye. **B**, Evaluation of PCO in the pseudophakic eye shown in A. **C**, Slit-lamp retroillumination photograph of an aphakia eye. **D**, Evaluation of PCO in the aphakia eye shown in C.

APPENDIX 2. SUMMARY OF ALL THE 41 INFLAMMATORY FACTORS WITH THEIR MEASURED VALUES.

To access the data, click or select the words “[Appendix 2.](#)”

APPENDIX 3. COMPARISON OF INFLAMMATORY FACTORS IN AQUEOUS HUMOR BETWEEN MALE AND FEMALE CONGENITAL CATARACT PATIENTS.

To access the data, click or select the words “[Appendix 3.](#)”

APPENDIX 4. COMPARISON OF INFLAMMATORY FACTORS IN AQUEOUS HUMOR BETWEEN BILATERAL AND UNILATERAL CONGENITAL CATARACT PATIENTS.

To access the data, click or select the words “[Appendix 4.](#)”

APPENDIX 5. SUMMARY OF POST-SURGERY INFLAMMATORY RESPONSES.

To access the data, click or select the words “[Appendix 5.](#)”

APPENDIX 6. CORRELATIONS BETWEEN INFLAMMATORY FACTORS AND THE POSTOPERATIVE INFLAMMATORY RESPONSE.

To access the data, click or select the words “[Appendix 6.](#)”

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