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Changes in aqueous humor cytokines and metabolomics in contralateral eye after unilateral cataract surgery

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Abstract

Background For patients with bilateral age-related cataracts, sequential phacoemulsification and intraocular lens implantation is a common treatment. However, it remains unclear whether surgery on the first eye has an impact on the second eye, as current research results are inconsistent. This study will explore whether surgery on one eye affects the non-operated eye through aqueous humor cytokines and metabolomic analyses in the second eye.

Methods A rabbit model of unilateral phacoemulsification and intraocular lens implantation was established. The experimental group consisted of 15 rabbits undergoing this procedure. Postoperatively, rabbits were divided into five subgroups (three rabbits per subgroup), and aqueous humor was collected from both the operated and non-operated eyes at 1 day, 3 days, 1 week, 2 weeks, and 3 weeks after surgery. Additionally, 5 rabbits were selected as a control group, from which aqueous humor was extracted. Levels of IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-8, IFN-γ, TNF-α, MCP-1, and VEGF in the aqueous humor were compared. In the clinical study, preoperative aqueous humor samples were collected from 22 patients undergoing bilateral phacoemulsification and intraocular lens implantation. Among them, 11 patients were tested for the aforementioned 10 cytokines, while the other 11 patients underwent untargeted metabolomics research.

Results In the animal experiment, levels of all 10 cytokines in the operated eyes were significantly higher compared to both the control and non-operated eyes groups ($P < 0.05$). In the non-operated eyes, IL-1β and IL-2 levels were also elevated compared to the control ($P < 0.05$); however, no statistically significant differences were observed between the non-operated eyes and the control group at postoperative time points of 1 day, 3 days, 1 week, 2 weeks, and 3 weeks. In the clinical study, no significant differences were found in cytokine levels between the two eyes. In the untargeted metabolomics analysis, 354 metabolites showed differential expression, 280 were upregulated and 74 were downregulated. Notably, Adenine and 2-Aminopurine were significantly downregulated, highlighting Purine metabolism as the most impacted pathway.

Conclusions Animal experiments showed a significant increase in IL-1β and IL-2 levels in the non-operated eyes postoperatively, reflecting systemic and local inflammatory responses. In clinical experiments, although no significant changes in cytokines were observed in the aqueous humor of both eyes, differential expression of metabolites indicated metabolic adjustments in the non-operated eye following surgery on the first eye. These findings reveal

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that unilateral cataract surgery may affect the stability of the intraocular environment in the contralateral eye, suggesting that in staged bilateral surgeries, potential metabolic changes in the non-operated eye and their clinical significance should be considered. This result provides important reference value for optimizing postoperative management strategies, reducing complications, and determining the timing for bilateral surgeries, warranting further investigation.

Keywords Metabolomics, Untargeted metabolomics, Diabetic retinopathy, Cataract, Aqueous humor, Mass spectrometry, Cytokine

Introduction

Age-related cataract (ARC) are the leading cause of blindness in ophthalmology, accounting for approximately 56.7% of all blinding eye diseases [1, 2]. Phacoemulsification combined with intraocular lens (IOL) implantation is the most common and effective method for treating ARC [2, 3]. ARC commonly affect both eyes. After cataract surgery on the first eye, differences in vision and refractive status between the two eyes can impact the patient's depth perception [4, 5], thus necessitating cataract surgery on the second eye for most patients within a short period [6]. Under topical anesthesia, patient cooperation is one of the key factors for the success of cataract surgery. The level of intraoperative pain directly determines the degree of patient cooperation [7, 8]. Clinical observations have found that in some patients who undergo cataract surgery on the second eye within a short period, there is often a noticeable increase in intraoperative eye pain compared to the first eye [9–13]. This leads to a decrease in patient cooperation [8], which may subsequently affect surgical procedures, intraoperative experience, and postoperative outcomes.Earlier discussions on this issue were more prevalent, but mostly focused on subjective psychological aspects, and the conclusions were not consistent. This phenomenon has been linked to reduced preoperative anxiety, which may make patients more relaxed during the second eye surgery [14–16]. However, the decrease in anxiety may lead to heightened awareness during the procedure, increasing sensitivity to pain and negatively impacting the experience of the second eye surgery. Additionally, this clinical phenomenon is associated with the patients' age, gender, and level of education [17]. However, some other teams reported opposite results, indicating that there was no difference in surgical pain and patient cooperation during the operation for both eyes [18]. With advances in molecular biology research, more teams are conducting detailed studies on this issue at the biochemical molecular level. In 2015, Xiang-Jia Zhu's team reported that levels of the inflammatory chemokine Monocyte Chemoattractant Protein-1 (MCP-1) in the aqueous humor (AH) significantly increased before the second eye cataract surgery. This chemokine is closely related to inflammatory responses and pain pathophysiology, suggesting that sympathetic ophthalmia (SO) may occur in the second eye after the first cataract surgery. This may help explain why the second eye phacoemulsification is typically more painful [19]. Subsequent studies by more teams have confirmed that MCP-1 levels are significantly higher in patients undergoing second eye surgery within a short period compared to the first eye [17, 20–22]. With ongoing research into this clinical issue, various teams have found that in patients undergoing sequential cataract surgeries, the aqueous humor of the second eye shows higher expression of additional biological factors such as Colony Stimulating Factor 3(CSF3) [23], IL-6, C–C Motif Chemokine Ligand 2(CCL2), IL-2, Macrophage Inflammatory Protein 1 delta(MIP-1d) [24], Tumor Necrosis Factor alpha(TNF-α), IL-1β [25, 26], and Transforming Growth Factor beta 2(TGF-β2) [27] after the first eye surgery.

Interestingly, in recent years, more and more teams have found that in non-diabetic patients, the aforementioned phenomenon and high expression of biological factors such as MCP-1 do not exist [28, 29].In diabetic patients, there is a significant increase in MCP-1 and substance P in the second eye [28, 29]. Furthermore, when different diseases are combined, the expression of biological factors in the aqueous humor before and after surgery varies among patients [30]. Therefore, in patients with systemic diseases, there are inherent differences in the AH environment.

Therefore, due to the current existence of different or even contradictory research results regarding this clinical issue, the purpose of this study is to investigate whether there are genuine changes in the AH of the second eye after the first eye surgery in patients with pure ARC. We strictly excluded cataract patients with other concurrent diseases and measured changes in the levels of AH biomarkers (IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-8, IFN-γ, TNF-α, MCP-1, and VEGF) in patients with pure ARC who underwent phacoemulsification and IOL implantation in both eyes, followed by targeted analysis. Additionally, we collected AH samples from healthy adult rabbits at different time intervals after surgery on the non-operated eye to observe changes in biomarkers over various time periods. Furthermore, we

utilized non-targeted metabolomics methods to analyze whether there are changes in the microenvironment of the AH in both eyes from a new perspective.

Metabolomics is a rapidly developing field within omics research in life sciences [31]. Metabolites, as downstream products of transcription, translation, and post-translational modification of proteins, can reflect various physiological processes [32]. Therefore, metabolomics research helps us better understand the pathophysiological processes of intraocular diseases. AH is a transparent intraocular fluid that maintains the metabolic processes of intraocular tissues and the normal homeostatic environment of the eye [33]. Compared to peripheral fluid samples such as blood, sweat, and urine, AH may better reflect local physiological changes related to intraocular diseases. Currently, there are no studies addressing the aforementioned clinical issues through AH metabolomics.

Liquid Chromatography/Mass Spectrometry (LC/MS) is a commonly used non-targeted metabolomics detection technique known for its high reliability and reproducibility [34]. Based on these characteristics, we applied ultra-high-performance liquid chromatography coupled with ultra-high-resolution mass spectrometry (UHPLC-OE-MS) to perform non-targeted metabolomics detection and analysis on AH samples from patients with pure ARC. This analysis aims to determine whether sequential cataract surgery in ARC patients affects the second eye following surgery on the first eye.

Materials and methods

Animal experimentation studies

This study adhered to the guidelines of the Declaration of Helsinki and was approved by the Animal Welfare and Ethics Committee of Zunyi Medical University (Approval No.: ZMU21-2203–590).

Inclusion criteria, grouping of animals

Twenty adult New Zealand white rabbits (1.8 − 2.2 kg; Yikeda Biotechnology Co., Ltd., Guizhou, China), both male and female, were used. The rabbits were divided into two groups using a random number table method: Group A ($n = 15$, right eye phacoemulsification combined with intraocular lens implantation) and the blank control group (Group B, $n = 5$, no surgery). Group A rabbits were further subdivided into five subgroups based on the postoperative time: 1 day, 3 days, 1 week, 2 weeks, and 3 weeks (each subgroup $n = 3$), labeled as A1d, A3d, A1w, A2w, and A3w, respectively. All animals were housed in the Laboratory Animal Resource Center at Zunyi Medical University, with a controlled environment (temperature 25 ± 1 °C, humidity 55% − 75%), and were provided with the same food and water.

Experimental procedure

Three days before surgery, both eyes of the rabbits were instilled with levofloxacin eye drops (5 $g \cdot L^{-1}$; Santen Pharmaceutical Co., Ltd., Tokyo, Japan) four times a day. The phacoemulsification combined with IOL implantation surgery was performed by the same experienced surgeon. Thirty minutes before surgery, tropicamide eye drops (Shenyang Xingqi Pharmaceutical Co., Ltd.) were instilled three times for pupil dilation, and 10 min before surgery, 2 drops of proparacaine hydrochloride ophthalmic solution (Nanjing Ruinian Best Pharmaceutical Co., Ltd., China.) were instilled for surface anesthesia. Intramuscular injection anesthesia with 0.3 $mL \cdot kg^{-1}$ of ketamine hydrochloride injection (Dunhua Shengda Animal Medicine Co., Ltd.) was administered. Routine disinfection of the surgical field was performed. Sterile drapes were applied, eyelid speculum was used to open the eyelids, povidone-iodine solution (Shanghai Lekang Disinfection High-Tech Co., Ltd.) was instilled into the eyes, and the conjunctival sac was rinsed with saline. Aqueous humor samples were collected from the anterior chamber by puncturing the cornea at the corneal side incision using a 29-gauge insulin syringe, collecting 150–200 μL of AH, and immediately storing it in a −80 °C freezer.

Phacoemulsification combined with IOL implantation: In the experimental group, after AH extraction, balanced salt solution was injected into the anterior chamber through the self-puncture site to restore anterior chamber depth. A transparent corneal incision was made 2.2 mm above the cornea, and viscoelastic material was injected into the anterior chamber. A circular capsulorhexis with a diameter of 5 mm was performed, followed by nucleus emulsification with phacoemulsification and cortical aspiration. IOLs (including SOFTEC I by Lenstec (Barbados) and SN6CWS by Alcon Laboratories) were implanted, and the corneal incisions were watertight. After surgery, the operated eyes were treated with tobramycin-dexamethasone ointment (Alcon, USA) and then placed back into the Laboratory Animal Resource Center for normal feeding upon awakening. Postoperative medication included tobramycin-dexamethasone eye drops in the operated eyes four times a day for the first week, followed by a reduction of one drop per week until discontinued after 4 weeks.

In the experimental groups (A1d, A3d, A1w, A2w, A3w), AH samples were collected from the operated eye (right eye) and the non-operated eye (left eye) on the corresponding days. For the control group, AH samples were randomly collected from both eyes on random dates. Prior to AH collection, disinfection was performed using povidone-iodine, and surface anesthesia was achieved with proparacaine hydrochloride ophthalmic solution (Nanjing Ruinian Best Pharmaceutical Co., Ltd., China.).

A 30-gauge sterile needle attached to a syringe was used to puncture the anterior chamber from the corneal limbus at a distance of 0.5 mm from the temporal side to extract 100–200 μl of AH samples. The collected samples were immediately placed in a −80 °C freezer for low-temperature preservation.

Measurement of animal cytokine concentrations

Total protein was extracted from rabbit AH using the concentrations sandwich enzyme-linked immunosorbent assay (cELISA). cELISA kits (provided by Hengyuan Biotech) were utilized to measure the concentrations of various biomarkers in AH, including cytokines: interleukins IL-1a, IL-1β, IL-2, IL-4, IL-6, IL-8, interferon-γ (IFN-γ), and TNF-α; chemokine: MCP-1; and growth factor: vascular endothelial growth factor (VEGF). The procedures were conducted according to the manufacturer's instructions. Absorbance was read at a wavelength of 450 nm using an enzyme-linked immunosorbent assay reader (Multiskan MS, Labsystems, Finland). The same procedures were followed for clinical samples.

Clinical Research

This prospective, single-blinded, randomized study was conducted from November 2023 to May 2024 at the Refractive Cataract Treatment Center of the Affiliated Hospital of Zunyi Medical University and the Ophthalmology Department of the Second Affiliated Hospital of Zunyi Medical University. Ethical approval was granted by the Ethics Committee of the Second Affiliated Hospital of Zunyi Medical University (Approval No.: KYLL-2023–013), and the study adhered to the principles of the Declaration of Helsinki. All patients signed written informed consent forms.

Human subject grouping and inclusion/exclusion criteria

The study recruited elderly patients with ARC who underwent sequential bilateral cataract phacoemulsification with IOL implantation surgery at the Refractive Cataract Treatment Center of the Affiliated Hospital of Zunyi Medical University and the Ophthalmology Department of the Second Affiliated Hospital of Zunyi Medical University from November 2023 to May 2024. All surgeries were performed under local anesthesia. The second eye surgeries for all patients were completed within 2 weeks after the first eye surgery. The eye with more severe cataract or poorer visual acuity was selected for the first eye surgery. Strict exclusion criteria were applied, including: (1) Tumor patients or patients with coronary heart disease who are taking non-steroidal anti-inflammatory drugs; (2) Patients with a history of eye trauma or eye surgery; (3) Patients with poor cooperation or unwillingness to undergo cataract surgery under local anesthesia; (4) Patients with baseline eye pain (including glaucoma or high intraocular pressure); (5) Patients currently taking painkillers or receiving pain treatment; (6) Patients with complicated cataracts; (7) Patients with hypertension or diabetes; (8) Patients with autoimmune diseases such as rheumatoid arthritis; (9) Patients with any other eye diseases (such as high myopia, corneal diseases, infectious eye diseases, choroidal and retinal diseases, etc.). Ultimately, 22 patients met the criteria and were included in the study analysis. Due to limitations in aqueous humor sample volume, 11 patients were randomly selected for measurement of biomarker concentrations in AH before surgery in both eyes, while another 11 patients underwent non-targeted metabolomics analysis. The baseline characteristics of the patients are presented in Table 1.

The surgical procedure and specimen collection in humans

All patients received levofloxacin eye drops (5 g·L-1) from Santen Pharmaceutical Co., Ltd., Tokyo, Japan, administered four times daily in both eyes for three days prior to surgery. Local anesthesia was typically administered using proparacaine hydrochloride ophthalmic solution (Nanjing Ruinian Best Pharmaceutical Co., Ltd., China.) at the beginning of the procedure. Subsequently, an eyelid speculum was used to keep the eye open and positioned under the surgical microscope. The conjunctival sac was alternately rinsed with povidone-iodine and copious amounts of saline solution. Following these steps, approximately 100–200 μl of aqueous humor samples were obtained from the anterior chamber using a 1-ml syringe through the transparent corneal incision above the anterior chamber angle membrane during both the first and second eye surgeries and immediately stored in a −80 °C freezer. Subsequently, conventional hydrodissection, phacoemulsification, nuclear fragmentation, and rotation were performed. The optimal intraocular lens was then implanted using a specialized injector. Finally, the incision was hydrated with balanced saline solution, and water tightness was ensured after removing any residual gel-like material. All surgeries for both eyes of each patient were performed by the same experienced cataract surgeon.

Measurement of human biological factor concentrations

Measurement of animal biomarkers as described above.

Chromatography-mass spectrometry acquisition

Metabolite extraction Each AH sample (50 μL) was transferred to a centrifuge tube. After adding 200 μL of extraction solution (acetonitrile: methanol = 1:1, containing isotopically labeled internal standard mixture; detailed information of the internal standard can be found in Attachment 5), the samples were sonicated in an ice

Table 1 Baseline characteristics of patients with age-related cataract undergoing bilateral cataract surgery

Patients characteristics	Cytokine analyses	Chromatography-mass spectrometry acquisition
Gender		
Female	7 (63.6%)	3 (27.3%)
Male	4 (36.4%)	8 (72.7%)
Age at surgery (median, range) (years)	68(50–89)	73 (61–91)
Site of second surgery	11(100%)	11(100%)
The first eye,OD/OS	5/6	9/2
Axial length (median, range) (mm)		
First eye	23.22(21.71–24.37)	23.36(22.52–24.44)
second eye	23.11(21.48–24.39)	23.32(22.50–24.81)
Interval time of surgery(median,range)(days)	7(1–13)	3(2–13)
Preoperative intraocular pressure of the right eye(median,range)(mmHg)	17.64(15–20)	17.55(12–21)
Preoperative intraocular pressure of the left eye(median,range)(mmHg)	17.55(14–20)	17.91(15–20)

bath for 10 min, followed by incubation at −40 °C for 1 h. Subsequently, the samples were centrifuged at 4 °C and 12,000 rpm (relative centrifugal force = 13,800 × g, R = 8.6 cm) for 15 min. The supernatant was transferred to a new glass vial for analysis. The supernatants of all samples were mixed in equal volumes to prepare quality control (QC) samples.

LC–MS/MS analysis LC–MS/MS analysis was performed using a Vanquish ultra-high-performance liquid chromatography system (Thermo Fisher Scientific) from Biotree Biotechnology Co., Ltd. (Shanghai, China). The target compounds were chromatographically separated using a Waters ACQUITY UPLC BEH Amide column (2.1 mm × 50 mm, 1.7 μm). The mobile phase A consisted of water with 25 mmol/L ammonium acetate and 25 mmol/L ammonia solution, while mobile phase B consisted of acetonitrile. The sample tray temperature was maintained at 4 °C, and the injection volume was 2 μL.

An Orbitrap Exploris 120 mass spectrometer was employed for data acquisition, controlled by Xcalibur software (version: 4.4, Thermo). The detailed parameters were as follows: sheath gas flow rate: 50 Arb, auxiliary gas flow rate: 15 Arb, capillary temperature: 320 °C, full MS resolution: 60,000, MS/MS resolution: 15,000, collision energy: SNCE 20/30/40, spray voltage: 3.8 kV (positive) or −3.4 kV (negative).

Data pre-processing and annotation The raw data was converted to mzXML format using ProteoWizard software. Metabolite identification was performed using a collaborative R package with the BiotreeDB (V3.0) database. Subsequently, visualization analysis was conducted using a custom R package.

Development of the diagnostic metabolic model The statistical analysis of the results was conducted using orthogonal projections to latent structures-discriminant analysis (OPLS-DA) to assess inter-group differences and the relevance to the experimental group [35]. Combined univariate and multivariate statistical analyses were performed to identify differential metabolites [36]. In the OPLS-DA model, the variable importance in the projection (VIP) values was calculated. Student's t-test was employed to compute the *p*-value for individual dimensions for analysis. Metabolites were considered statistically significant when VIP > 1 and $P < 0.05$. Metabolic pathway enrichment and pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database were utilized to summarize and map the biochemical pathways associated with the differences between the two groups of metabolites. Upon obtaining matching information for the differential metabolites, pathway databases for the corresponding species Homo sapiens (human) were searched, and metabolic pathway analysis was performed. By comprehensively examining pathways containing differential metabolites (including enrichment analysis and topological analysis), pathways were filtered, and the key pathways most closely related to metabolite differences were identified.

Statistical analyses

Biological factor data statistical analysis

All data were statistically analyzed using SPSS 29.0 (IBM-SPSS, Armonk, New York, USA). In the clinical study, differences were compared using independent samples t-tests. In the animal experiment section, based on strict normality, an independent samples t-test was used to compare the right and left eyes of the experimental

rabbits with the control group. For the experimental group, differences in significance between the subgroups at 1 day, 3 days, 1 week, 2 weeks, and 3 weeks post-surgery and the control group were compared using independent t-tests, provided that the data met the normality assumption. Additionally, the Holm-Bonferroni method was applied to assess the potential for Type I error. A bilateral P-value < 0.05 was considered statistically significant.

Statistical analysis of differential metabolites

PCA and OPLS-DA were employed to visualize global metabolic differences between groups. PCA was performed using SIMCA software (V16.0.2, Sartorius Stedim Data Analytics AB, Umea, Sweden), where the data underwent logarithmic (LOG) transformation and centering (CTR) formatting before conducting automated modeling analysis [37]. The raw data was converted to mzXML format using ProteoWizard software, and metabolite identification was conducted using a collaboratively developed R package. The database utilized for this purpose was BiotreeDB (V3.0) [38].

Then, visualization analysis was performed using a self-developed R package. Pathway enrichment analysis was conducted on MetaboAnalyst (http://www.kegg.jp/kegg/pathway.htm) to obtain significantly enriched KEGG pathways. We searched and analyzed pathway regulation networks in the KEGG database for the corresponding species Homo sapiens (human). Matchstick plots were created using the R ggplot2 package to identify quantitative differences in differential metabolites. Receiver Operating Characteristic (ROC) curves were generated using the R plotROC and pROC packages to demonstrate predictive ability. The R pheatmap package was utilized to classify metabolites with similar features. Statistical analysis was performed using R versions 16.0.2, 3.3.5, 2.2.1, and 1.16.2.

Results

Analysis of cytokines in the aqueous humor of rabbits

To explore potential changes in the contralateral eye's AH inflammatory factors, we collected AH samples from both the operated and non-operated eyes at various postoperative time points following the initial eye cataract surgery. We employed a cELISA to measure the concentrations of ten selected inflammatory factors. All statistical descriptions and results for these inflammatory factors are summarized in Supplementary Tables 2 and 3. Remarkably, all inflammatory factors exhibited significantly higher levels in the operated eye group compared to both the non-operated eye group and the control group. However, when comparing the non-operated eye group with the control group, IL-1β ($p = 0.043$) and IL-2 ($p < 0.001$) demonstrated increased levels. However, at postoperative time points of 1 day, 3 days, 1 week, 2 weeks, and 3 weeks, no significant statistical differences were observed between IL-1β and IL-2 in the non-operated eye group compared to the control group. Nevertheless, the trend in concentration changes over time indicated a decrease in both cytokines starting at 2 weeks post-surgery. Figures 1 and 2 shows the concentration distribution of the 10 cytokines in the control group, operated eye group, and non-operated eye group, along with their statistical significance, while Figs. 3 and 4 illustrates the comparison of cytokine concentrations at different postoperative time points between the operated eye group, non-operated eye group, and control group.

Analysis of patient's aqueous humor cytokines

According to strict inclusion criteria, concentrations of the ten Cytokines analyzed in AH samples collected from both eyes of patients before cataract surgery showed no statistically significant differences. Bilateral AH concentration detection is shown in the forest plot (Fig. 5).

The non-targeted metabolomic results of human aqueous humor.

Overview of data quality

We monitored instrument stability and ensured data quality by assessing the differences in peak heights of internal standards across QC samples. As shown in Fig. 6A and B, all QC samples exhibited good overlap of retention times and peak areas for internal standards in both positive and negative ion modes, indicating excellent stability of response intensity. The detection of substance residues throughout the detection process was examined by analyzing blank samples interspersed throughout the experiment. As depicted in Fig. 6C and D, no significant peaks were detected for any internal standards in all blank samples, indicating effective control of substance residues and manageable cross-contamination between samples.

Principal component analysis (PCA) was performed on the features detected in the second-eye group and first-eye group (control group) samples. Quality control samples were tightly clustered together in both positive and negative ion modes (Fig. 7A and B), indicating good reproducibility and high reliability of the data in this experiment.

Establishing an OPLS-DA model provided more reliable information regarding the intergroup differences in metabolites and their correlation with the experimental groups. As depicted in the OPLS-DA score plot (Fig. 8A), the separation between the First-eye surgery group and the Second-eye surgery group is well-defined. The validation plot (Fig. 8B) shows that the R2Y(cum) values are

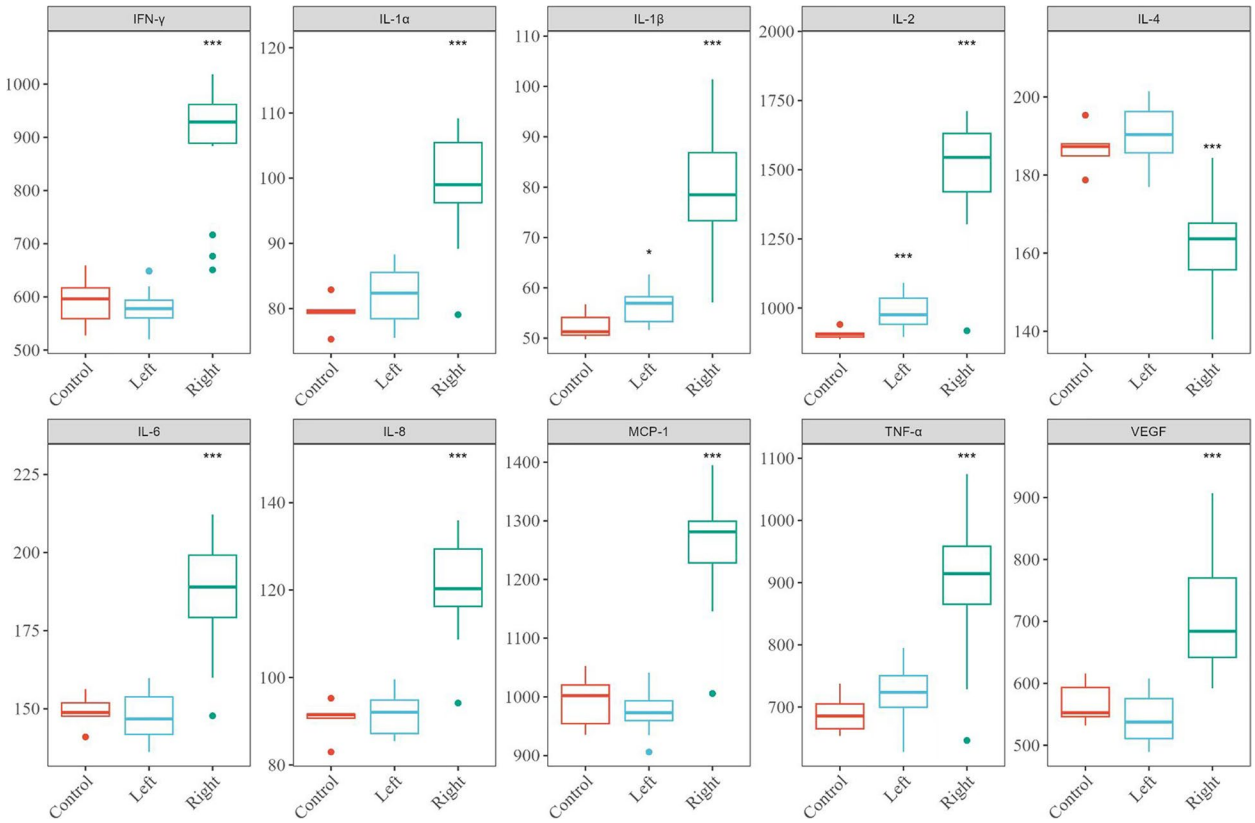

Fig. 1 Box plots illustrating the concentrations of ten cytokines (IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, MCP-1, TNF-α, and VEGF) across three groups: Control, Left (non-surgical eye), and Right (surgical eye). The x-axis denotes the groups, while the y-axis represents cytokine concentration levels. Statistical significance is indicated by asterisks: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

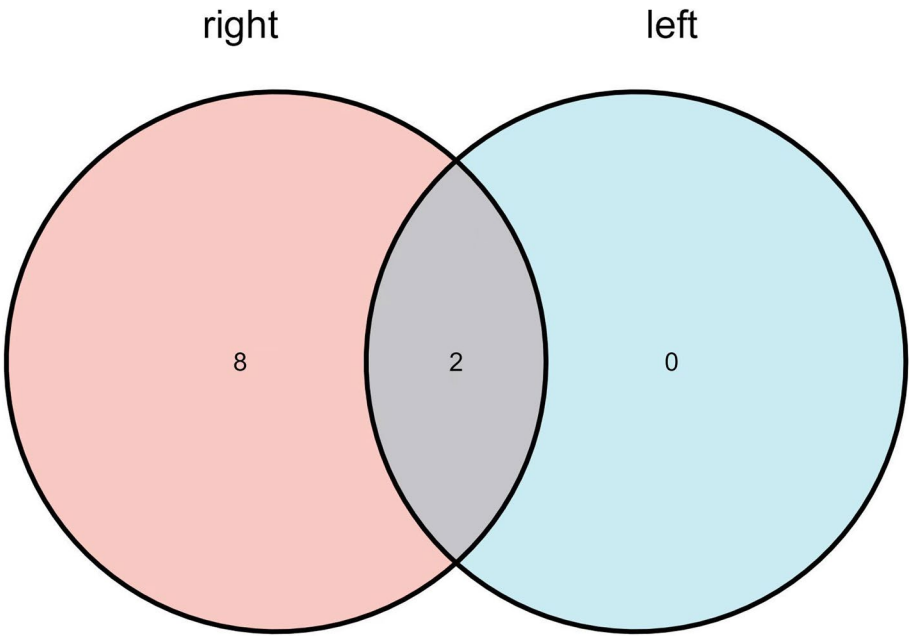

Fig. 2 Venn diagram illustrating the statistically significant differences in cytokine types between the right eye group, left eye group, and control group. The comparison between the right eye group and the control group revealed significant differences in all ten cytokines, while the comparison between the left eye group and the control group showed significant differences in two cytokines

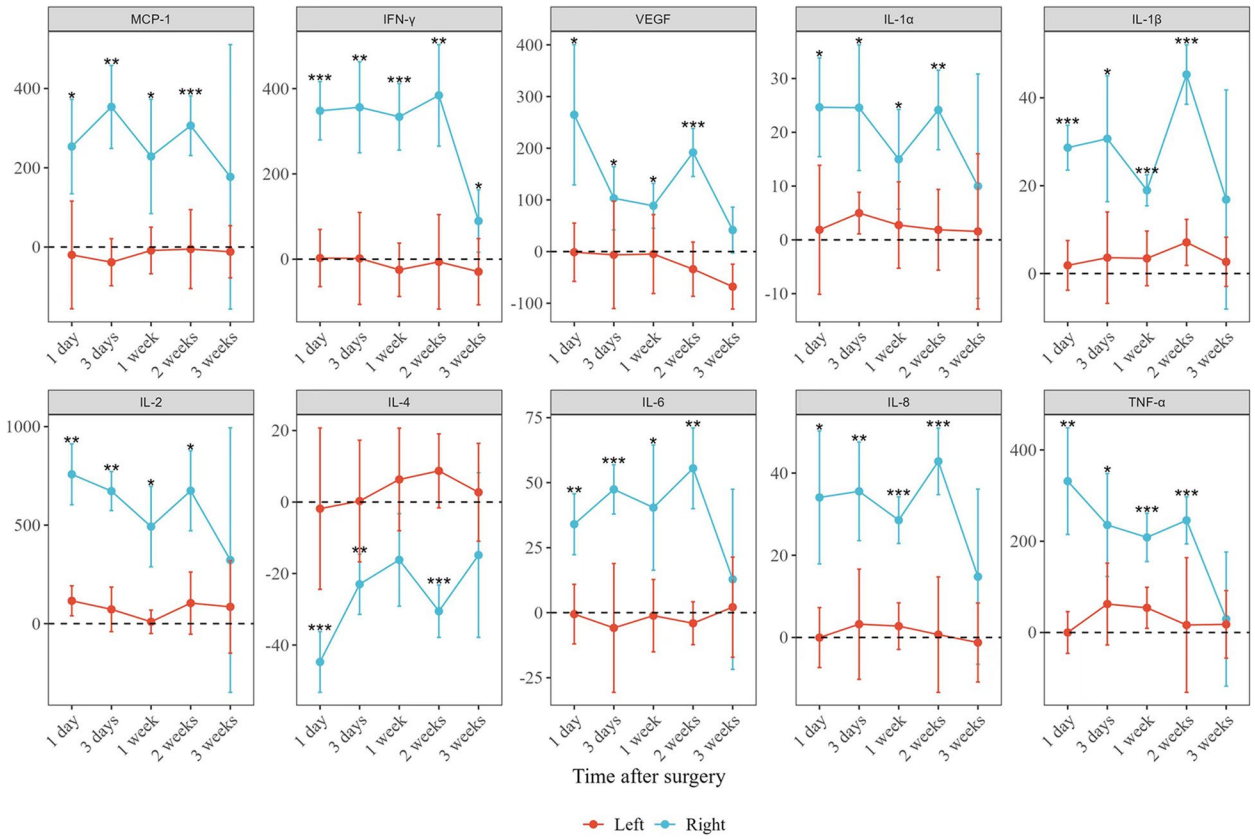

Fig. 3 Line graph of the concentration changes comparing the operated eye (right eye), non-operated eye (left eye), and control group. The vertical axis represents cytokine concentration, and the horizontal axis represents time. Asterisks indicate statistical significance compared to the control group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

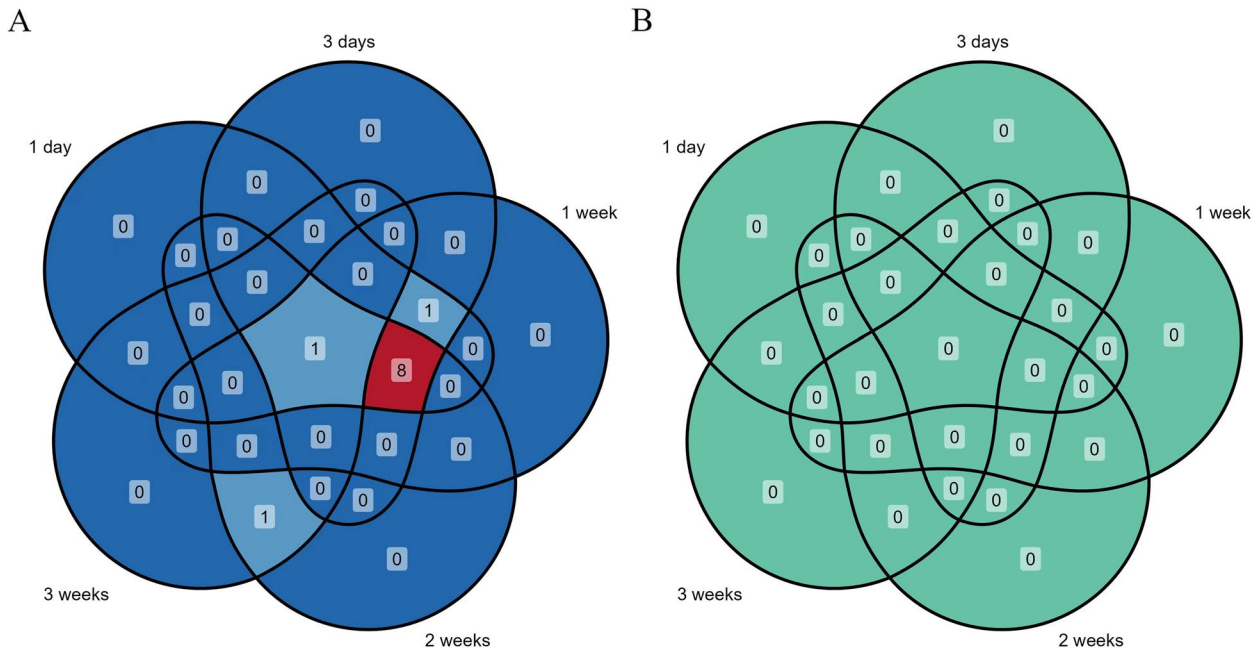

Fig. 4 Venn diagram showing the cytokines with statistically significant differences between the right eye group (**A**, operated eye) and left eye group (**B**, non-operated eye) compared to the control group at five postoperative time points: 1 day, 3 days, 1 week, 2 weeks, and 3 weeks

Indicator	First eye	Second eye	Mean difference (95% CI)	P value
MCP-1	276.23 ± 37.95	276.73 ± 40.50	-0.50 (-11.28, 10.28)	0.919
VEGF	1149.51 ± 210.52	1140.06 ± 230.89	9.46 (-45.53, 64.44)	0.710
IL-1α	26.26 ± 4.81	27.14 ± 4.21	-0.88 (-2.49, 0.72)	0.248
IL-1β	43.72 ± 8.06	43.80 ± 7.71	-0.08 (-2.28, 2.12)	0.935
IL-2	149.81 ± 19.03	149.78 ± 19.25	0.03 (-7.13, 7.18)	0.993
IL-4	500.49 ± 120.31	511.14 ± 126.40	-10.65 (-32.78, 11.48)	0.309
IL-6	18.20 ± 4.53	17.90 ± 3.80	0.30 (-0.88, 1.49)	0.584
IL-8	1332.99 ± 150.65	1344.14 ± 209.48	-11.14 (-78.68, 56.39)	0.721
IFN-γ	424.04 ± 78.18	431.79 ± 86.70	-7.76 (-35.66, 20.15)	0.549
TNF-α	444.36 ± 63.75	457.03 ± 56.54	-12.67 (-33.58, 8.24)	0.207

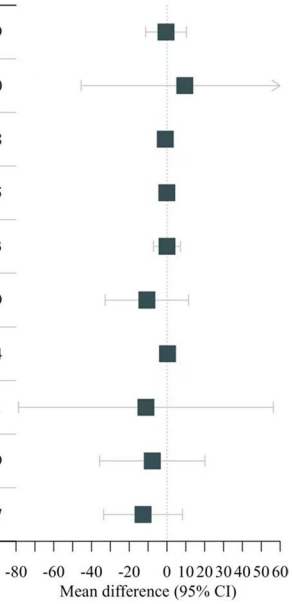

Fig. 5 Forest plot of cytokine levels in the aqueous humor of the first and second eyes

close to 1, indicating a strong explanatory power of the model towards the data, effectively explaining the relationship between variables and categorical variables. The lowest Q2(cum) value is still 0.38, suggesting a certain predictive capability of the model. Additionally, the results of the permutation test are shown in the figure, with the blue squares forming a gradually decreasing trend line, indicating that the predictive ability of the model is not due to data overfitting but rather exhibits a certain robustness.

Differential metabolic profile of AH Between First-Eye and Second-Eye Surgery Groups

By combining univariate and multivariate statistical analysis results, we identified differential metabolites. This approach not only helps us observe the data from different perspectives to draw conclusions but also assists in avoiding false positives or model overfitting that may arise from using only one type of statistical analysis method [36].

A total of 14,581 metabolites were detected in both the first eye and second eye groups, with 354 differential metabolites identified, consisting of 280 upregulated and 74 downregulated metabolites. To ensure the reliability of the identified differential metabolites, we applied filtering criteria based on VIP values greater than 1 and Student's t-test *P*-values less than 0.05. Additionally, manual inspection was conducted to exclude exogenous compounds such as drugs and chemical contaminants. Consequently, 22 differential metabolites were determined, including 17 upregulated and 5 downregulated metabolites. Specifically, the downregulated metabolites were identified as Adenine, 2-Aminopurine, SM(d34:1), L-Serine methyl ester, and 2-[(3,5-Dioxo-2,3,4,5-tetrahydro-1,2,4-triazin-6-yl)sulfanyl]propanoic acid. The upregulated metabolites included N-Acetyl-beta-D-glucosaminylamine, Oxooctatrienylcarnitine, Carbobenzyloxy-L-norvalyl-L-norleucine, N-alpha-methylhistamine, Polystachosid, among others. The final set of identified differential metabolites is illustrated in the matchstick plot (Fig. 9) and the cluster heatmap (Fig. 10). Furthermore, the ROC analysis of differential metabolites was conducted to select those with high accuracy for the comparison between the two groups, and the ROC curve plot was generated accordingly (Fig. 11).

Metabolic pathway analysis

To elucidate the metabolic pathways associated with the differential metabolites, we utilized the KEGG Pathway database (http://www.kegg.jp/kegg/pathway.html), which is based on functional information of genes and genomes and provides clues to metabolic reactions and regulatory proteins. We mapped all pathways related to the differential metabolites of the species Homo sapiens (human) and identified three pathways: Nucleotide metabolism—Homo sapiens (human), Purine metabolism—Homo sapiens (human), and Bile secretion—Homo sapiens (human). Among these pathways, Bile secretion—Homo sapiens was upregulated, while the other two pathways were downregulated. Specifically, in Nucleotide

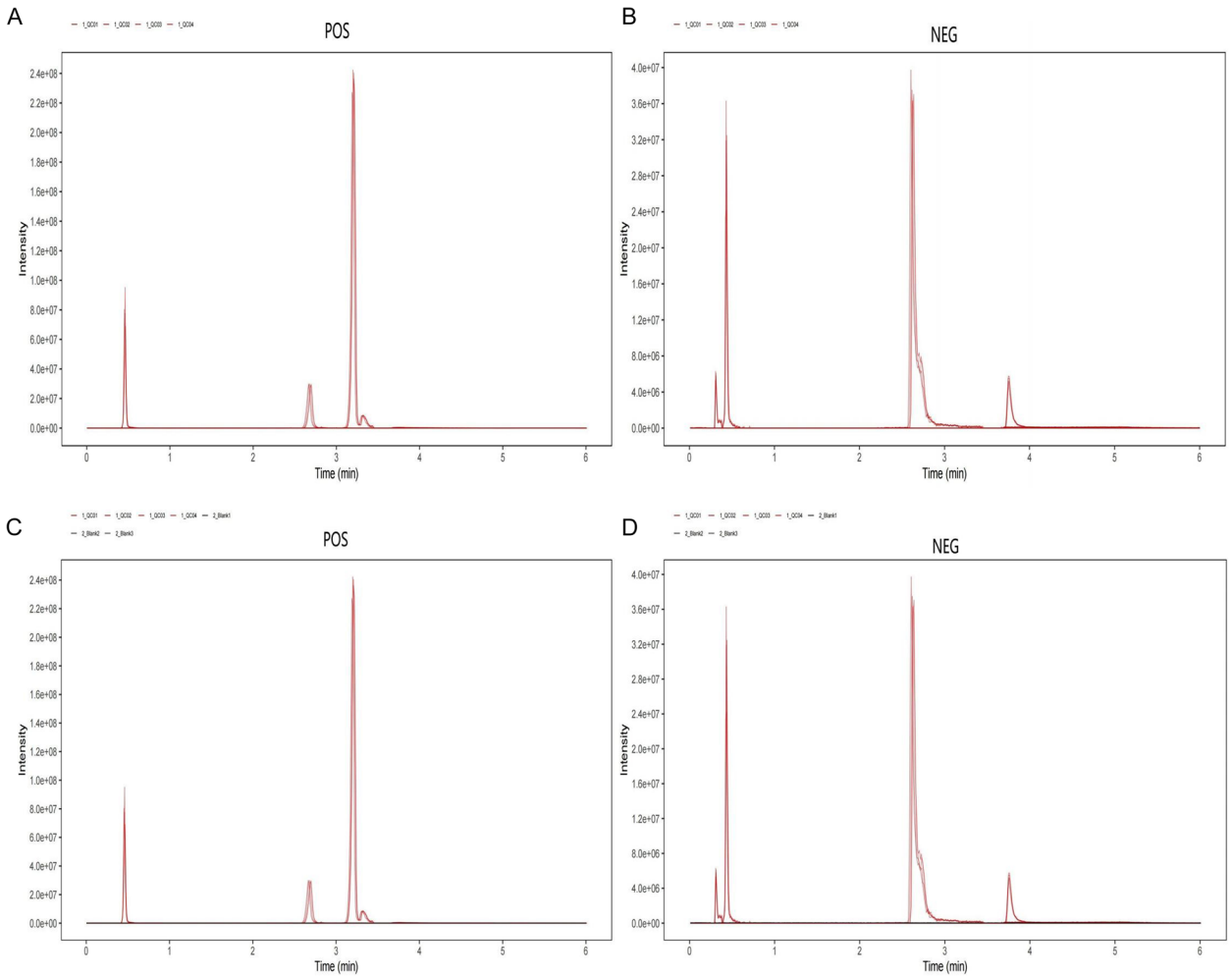

Fig. 6 QC assessment of UHPLC-OE-MS. Extracted ion chromatograms (EICs) of QC samples in positive ion mode (POS, **A**) and negative ion mode (NEG, **B**). EICs of blank samples and QC samples with internal standards (POS, **C**) and (NEG, **D**).The features detected in the samples of the second-eye group and the control group (first-eye group) were subjected to PCA. The QC samples were closely clustered together in both positive and negative ion modes (Fig. 6**A**, **B**), indicating good repeatability and high data reliability in this experiment

metabolism—Homo sapiens and Purine metabolism—Homo sapiens, only one differential metabolite, Adenine, was detected, and it was downregulated, as shown in Fig. 12.

Following comprehensive analysis of the pathways associated with the differential metabolites (including enrichment analysis and topological analysis) [39], we identified Purine metabolism as the pathway most closely associated with the differential metabolites.

Discussion

As mentioned earlier, the phenomenon of increased pain experienced by patients undergoing sequential bilateral cataract surgery during the second eye procedure has been widely studied for quite some time [11, 40]. From subjective responses or visual analog scale assessments [14, 18, 41, 42] to various biomarker expression or in vivo pain studies in animal experiments, it seems that each stage yields different, sometimes contradictory results. We selected ARC patients undergoing sequential surgeries within a short period, strictly adhering to inclusion criteria. We collected aqueous humor samples from both eyes preoperatively to investigate biomarkers and non-targeted metabolomics related to this issue, aiming for a comprehensive and objective exploration of this clinical problem at a more downstream level.

Our animal experiments indicate that all 10 biomarkers detected in the aqueous humor of the right eye (surgical eye) group showed a significant increase. The increased inflammatory response in the post-cataract surgery eye has been recognized by ophthalmologists, and our experimental results corroborate with previous findings [19, 43–45]. However, the significant increase observed in all 10 biomarkers compared to the control group and the

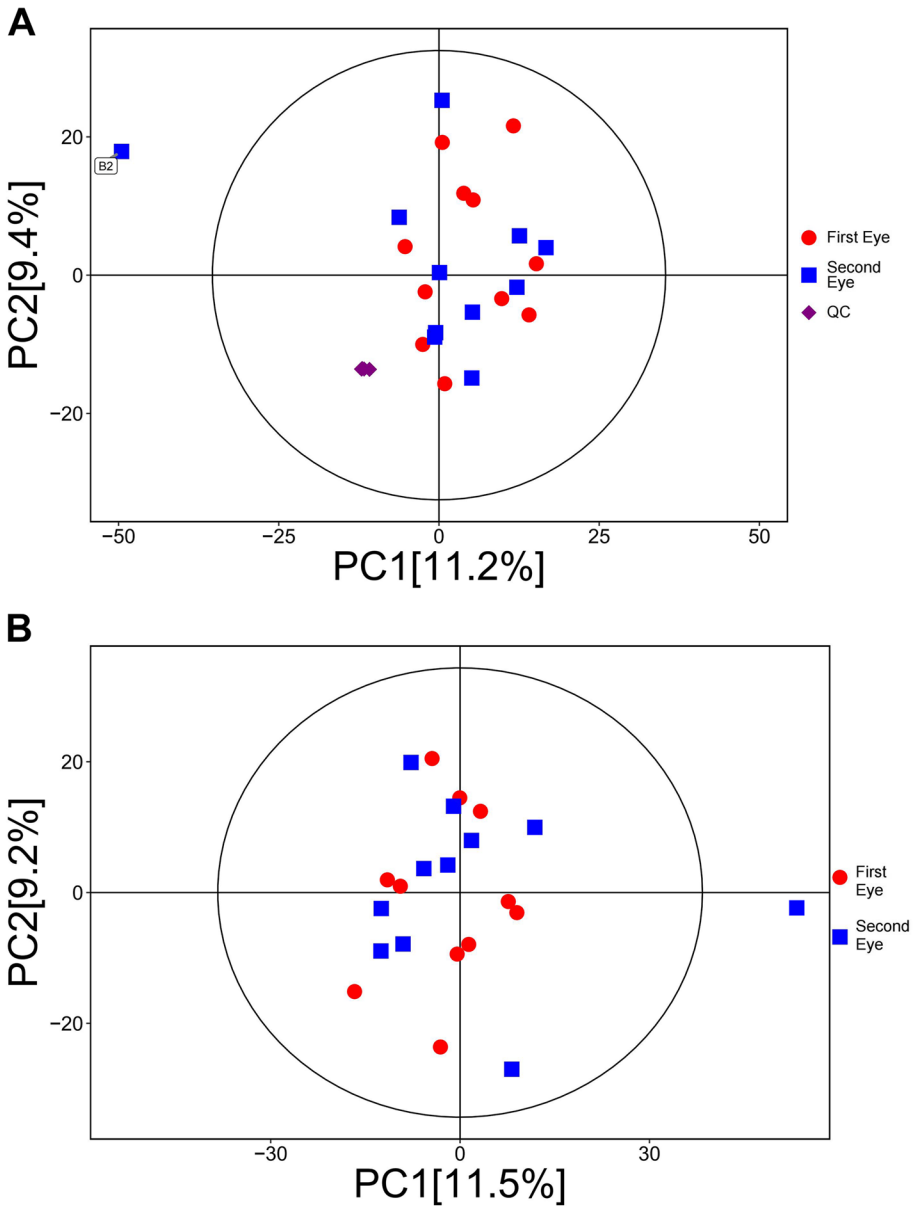

Fig. 7 PCA score scatter plots of all samples (including QC samples). As shown in Fig. 7**A**: The horizontal axis PC[1] and the vertical axis PC[2] represent the scores of the first and second principal components, respectively. Each data point represents a sample, with different colors and shapes indicating different groups. The closer the sample points are, the more similar the types and concentrations of metabolites in the samples. The samples are mostly within the 95% confidence interval (Hotelling's T-squared ellipse). Figure 7**B**. PCA score scatter plot of the First-eye group compared to the Second-eye group as shown in the figure: The samples are mostly within the 95% confidence interval (Hotelling's T-squared ellipse)

non-surgical eye group suggests that the local microenvironment changes and inflammatory responses post-cataract surgery far exceed our current understanding of the situation. It's worth noting that the AH microenvironment and immune function in rabbits are vastly different from those in humans. Moreover, our primary focus is on investigating the impact of surgery on the non-surgical eye, so we won't delve into this further here.

To better simulate cataract surgery in rabbits and verify the effects of cataract phacoemulsification and IOL implantation on the contralateral eye, we pioneered the implantation of IOL in the rabbit model. Postoperatively, we observed changes in IL-1β and IL-2 levels in the contralateral eye and further validated the expression patterns at different time points. However, no significant statistical differences were observed at the postoperative

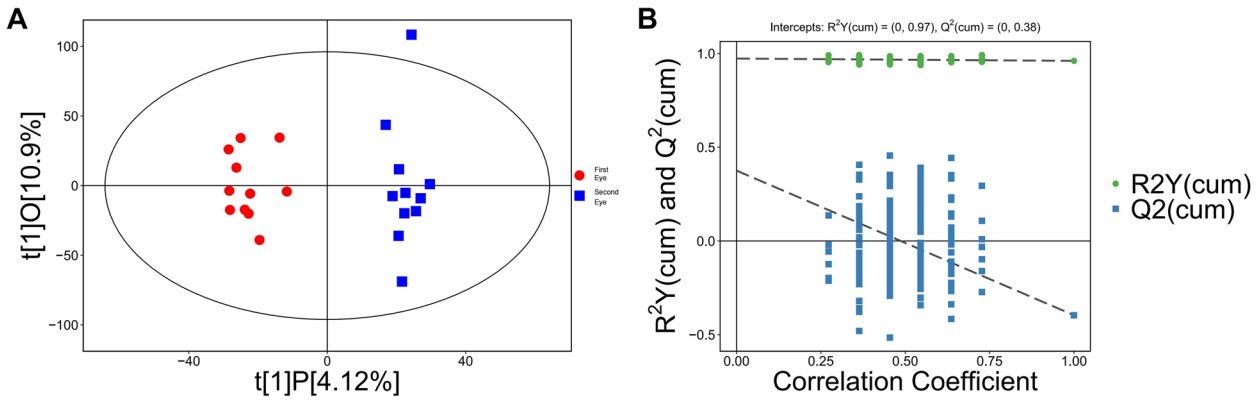

Fig. 8 **A** displays the OPLS-DA model score scatter plot for the First-eye group versus the Second-eye group. The horizontal axis represents t[1]P, which indicates the predictive component score of the first principal component, showing inter-group differences. The vertical axis represents t[1]O, indicating the orthogonal component score, illustrating intra-group differences. Each data point represents a sample, with different experimental groups represented by different colors and shapes. Larger horizontal distances between samples indicate greater inter-group differences, while smaller vertical distances suggest better intra-group repeatability. In Fig. 8**B**, the results of the permutation test for the OPLS-DA model between the First-eye group and the Second-eye group are presented. The horizontal axis represents the permutation retention rate of the permutation test (the proportion consistent with the original model Y variable order, with a retention rate of 1 indicating the RY and Q values of the original model). The vertical axis represents the values of RY or Q. Green circles represent the RY values obtained from the permutation test, while blue squares represent the Q values obtained from the permutation test. Two dashed lines represent the regression lines for RY and Q

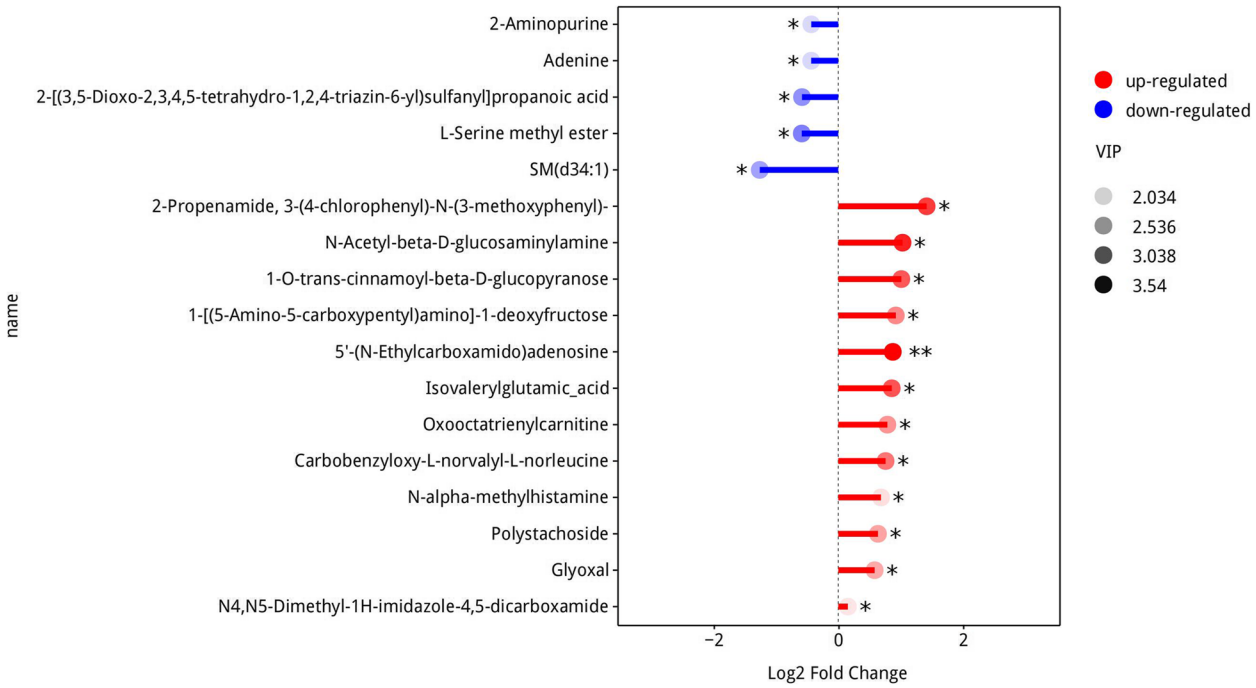

Fig. 9 Matchstick analysis of differential metabolites between the First eye and Second eye groups. Quantitative values of the differential metabolites were calculated for their corresponding ratios and subjected to logarithmic transformation with a base of 2. The top 12 upregulated and downregulated fold changes were selected for visualization. The x-axis represents the logarithmic transformed fold changes, while the color intensity of the points indicates the magnitude of the VIP values. This analysis showcases metabolites with significant changes, potentially reflecting strong activation or inhibition of corresponding enzyme gene expressions. Verification of the regulation of enzyme gene expressions related to these metabolites can be performed accordingly. * denotes significance. (Note: * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$)

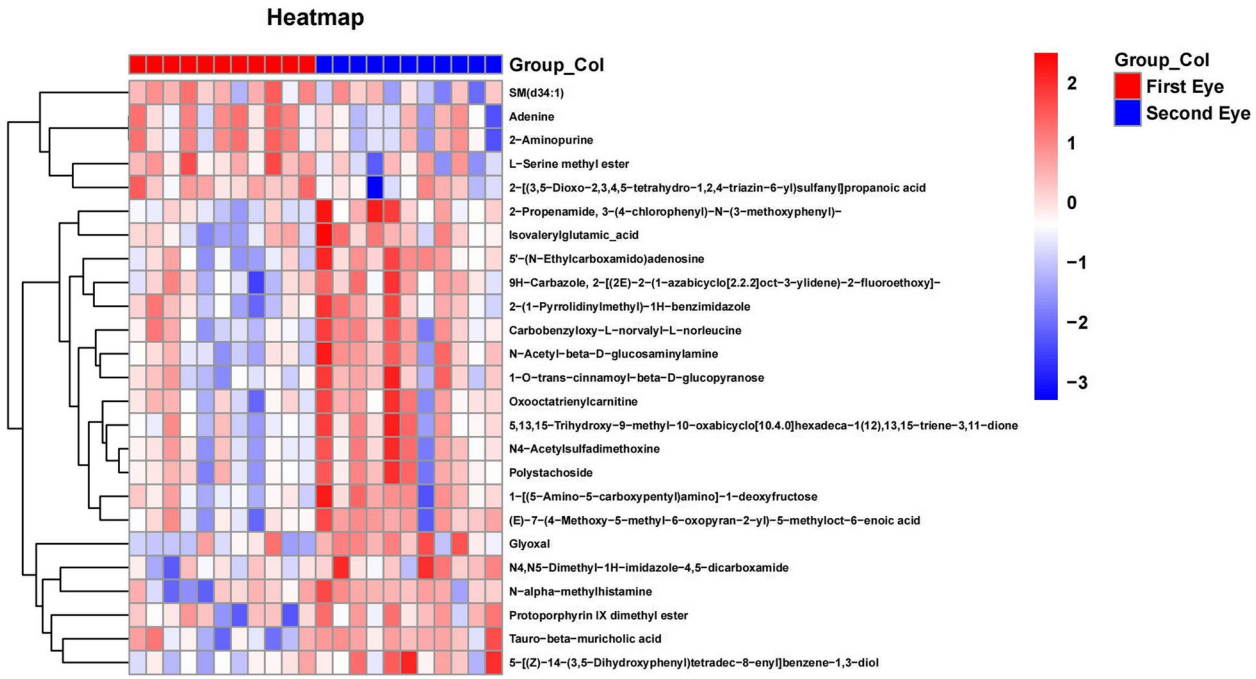

Fig. 10 Heatmap of hierarchical clustering analysis comparing the First eye and Second eye groups.The horizontal axis represents different experimental groups, while the vertical axis represents the differential metabolites compared between the groups. The color intensity at different positions in the heatmap represents the relative expression levels of the corresponding metabolites. Red indicates high expression levels of the metabolite in the respective group, while blue indicates low expression levels. (Note: **A** represents the First eye group, and **B** represents the Second eye group)

time points of 1 day, 3 days, 1 week, 2 weeks, and 3 weeks, which may be attributed to the limited sample size of the experiment. Nevertheless, the cytokine concentration trend revealed that IL-1β levels in the contralateral aqueous humor gradually decreased after 2 weeks postoperatively, consistent with the findings of Yang R et al. in their animal study.

Generally, Th17 cells are the main effector cells in the pathogenesis of inflammatory diseases, and IL-1β plays a crucial role in the differentiation and function of Th17 cells [46]. When activated by IL-1β, dendritic cells (DCs) express high or low levels of CD73 on γδ T cells, enabling them to either suppress or enhance adaptive immune responses [47]. The production of IL-1β is strictly regulated because abnormal activation can lead to chronic inflammatory diseases [48]. The expression of IL-1β is low in the central and peripheral nervous systems, but increases after injury. IL-1β can trigger the release of the final inflammatory mediators prostaglandin E2 and sympathetic amines, which can directly sensitize pain receptors [49]. VVerri Jr. et al. proposed that granulocyte colony-stimulating factor-induced hyperalgesia may be mediated by peripheral production of pro-algesic cytokines TNF-α and IL-1β [50]. Thus, IL-1β plays a crucial role in inflammation, modulating immune responses and enhancing pain sensitivity, contributing to the development of inflammatory diseases. These mechanisms may be key to understanding the inflammatory response and pain alterations in the contralateral eye after unilateral surgery.

IL-1β is released in response to various PAMPs (pathogen-associated molecular patterns) and DAMPs (damage-associated molecular patterns). The first eye cataract surgery can be considered as a form of damage, triggering the release of IL-1β. IL-1β lacks a signal sequence and does not follow the traditional protein secretion pathway but is secreted via one or more unconventional pathways [51]. The secretion pathway and concentration of IL-1β are determined by the intensity of the stimulus [51]. The release of IL-1β and its relationship with inflammation have been widely confirmed in numerous studies across various fields. Therefore, the elevated IL-1β levels in the second eye after the first eye surgery indirectly indicate the presence of an inflammatory response in the second eye. A substantial body of literature suggests that the first eye surgery, as a form of damage, triggers IL-1β release, which, through mechanisms such as the disruption of the blood-aqueous barrier [52] and sympathetic responses [53–55], leads to the elevated IL-1β levels in the aqueous humor of the second eye.

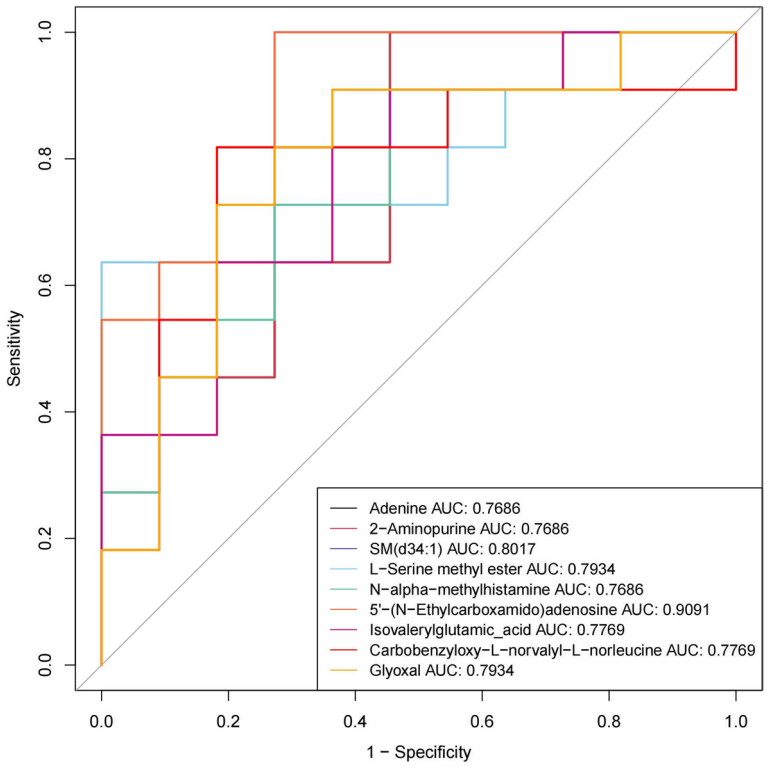

Fig. 11 ROC curve of differentially expressed metabolites between the First eye and Second eye groups.The area under the ROC curve (AUC) ranges between 1.0 and 0.5. A higher AUC value closer to 1 indicates a better diagnostic performance. AUC values between 0.5 and 0.7 suggest low accuracy, while values between 0.7 and 0.9 indicate moderate accuracy. AUC values above 0.9 indicate high accuracy

Through the analysis of AH in patients with diabetes, high myopia, and primary angle-closure glaucoma, Jiancen Tang and colleagues discovered that IL-2 levels in the aqueous humor of the contralateral eye increased compared to the preoperative levels in the operated eye after surgery. This phenomenon was also confirmed in our animal experiments [30]. It is well known that IL-2, produced by T cells, is an immunoregulatory protein [56]. IL-2 plays a crucial role in regulating the immune system, particularly in the proliferation, differentiation, and function of T cells. Its impact on the immune system is multifaceted [56]. Firstly, IL-2 can stimulate the proliferation and activation of T cells, promoting their differentiation into various types of effector T cells, such as cytotoxic T lymphocytes (CTLs) and regulatory T cells (Tregs). Secondly, IL-2 can enhance the activity of both itself and other immune cells, including natural killer (NK) cells, B cells, and macrophages, thereby strengthening the immune response. Additionally, IL-2 can regulate immune tolerance, which is the immune system's ability to recognize and not attack self-tissues, thereby preventing the occurrence of autoimmune diseases [19, 57, 58]. It has been demonstrated that IL-2 can amplify regulatory T cells in various diseases, and these amplified T cells can suppress excessive inflammatory responses, thereby exerting a protective effect [58–60]. In this experiment, the observed increase in IL-2 levels in the non-operated eyes of rabbits appears to be a protective response, aimed at mitigating the systemic and sympathetic inflammatory activation induced by the surgery (see Fig. 13).

This phenomenon, where surgery on one eye leads to pathophysiological changes in the contralateral eye, appears to represent a subclinical state of sympathetic ophthalmia [19]. In his initial description, Mackenzie hypothesized that the most likely mechanism for the spread of inflammation from one eye to the other is through the optic nerve and optic chiasm [61]. An increasing body of evidence supports the role of the immune system in ultimately leading to SO in the other eye [53, 62, 63]. Cataract surgery is one of the procedures associated with the development of SO [54, 55]. Our animal experiments, which detected high expression levels of inflammatory factors, also validate this state of immune alteration [56].

Unfortunately, in our study of AH biomarkers in patients undergoing short-term phacoemulsification cataract extraction combined with IOL implantation in both eyes, we did not find any statistically significant

differences in biomarkers. This finding appears contradictory to the study by Zhu X-J et al. [19, 20, 27]. However, recent more in-depth research on this issue, especially when carefully distinguishing whether cataract patients have comorbidities such as diabetes or other systemic and ocular conditions, has shown that in patients with uncomplicated senile cataracts, no significant increase in biomarkers compared to the control group has been observed [28, 30]. In contrast, elevated expression of biomarkers such as MCP-1 in the aqueous humor of the non-operated eye has been found in patients with comorbidities such as diabetes and high myopia. Hong Yan and others similarly confirmed in their study of patients with congenital cataracts that surgery on the first eye does not lead to changes in cytokines in the contralateral eye [29]. From an age perspective, patients with congenital cataracts appear to have a lower likelihood of comorbidities such as diabetes, hypertension, or acute glaucoma attacks. However, why do different research teams yield inconsistent or widely differing results when performing the same surgery? We consider: 1. Differences in biomarker detection methods may lead to discrepancies in detection results due to variations in sensitivity and concentration requirements. 2. Inconsistent inclusion criteria and potential errors in clinical case collection, as cataract surgeries are often outpatient procedures with significant regional differences in patients' cultural backgrounds, knowledge levels, and health awareness. Some patients with conditions like hyperglycemia or other systemic diseases may go undetected, introducing biases in medical history collection. 3. Variability in cataract severity and surgical procedures among different patients and surgeons may affect surgical trauma, duration, and subsequent local and systemic immune responses, resulting in inconsistent inflammatory states in non-operated eyes [64]. 4. The complexity of individual immune responses, leading to varying reactions to the same surgical trauma. 5. Whether patients receive analgesics, sedatives, or other medications after the first eye surgery, potentially affecting immune responses and test outcomes.

We know that there are complex interactions between cytokines and metabolites. They play crucial roles in maintaining homeostasis, regulating immune responses, transmitting signals, and other physiological functions within the body [65]. In our clinical study, we did not find differences in the expression of biomarkers between the two eyes. Therefore, we further conducted metabolomics research to analyze downstream whether there are actual changes in internal homeostasis and function in the non-operated eye. Among the differential metabolites, we found significant downregulation of adenine and 2-aminopurine, which were the top matching metabolites based on scoring (see Supplementary Table 6: Differential Metabolite Expression Products). Pathway analysis of differential metabolites revealed that purine metabolism is the key pathway most associated with the differences in metabolites. These findings suggest that abnormalities in adenine metabolism play a role in the impact on both eyes after surgery on one eye.

We know that purine metabolism is a fundamental pathway in human metabolism. Complex metabolic reactions and their regulation in organisms do not occur independently but rather form complex pathways and networks involving different genes and proteins. Their interactions and mutual regulation ultimately lead to systemic changes in the metabolome. Thus, from our differential metabolite analysis, differences in lipid, amino acid, and carbohydrate metabolites can be identified (see Supplementary Table 7: Classification of Differential Metabolites). Additionally, the regulation of inflammation also relies on strict control over the release and extracellular metabolism of purines. The expression and function of molecules related to purine release, metabolism, and signal transduction are typically induced in activated immune cells. Their activity is regulated by factors in the local environment such as bacterial toxins, hypoxia, and potassium ion concentrations [48]. Purine receptors are expressed in almost all immune cells. Peripheral enzymes such as CD39 and CD73 regulate immune responses by converting ATP into adenosine [66]. Therefore, the low expression of adenine not only affects ATP and DNA

(See figure on next page.)

Fig. 12 **A** KEGG Enrichment plot of differential metabolites between the First eye group and the Second eye group. The x-axis represents the Rich Factor of each pathway, while the y-axis indicates the names of KEGG metabolic pathways. The size of the circles indicates the number of differential metabolites enriched in each pathway. The color represents the significance level of the *p*-value, with a redder color indicating a more significant enrichment. Figure 12**B**. Differential Abundance Score plot for the First eye group vs. the Second eye group. The x-axis represents the Differential Abundance Score (DA Score), while the y-axis indicates the names of KEGG metabolic pathways. The DA Score reflects the overall change of all metabolites in the metabolic pathway, with a score of 1 indicating an upregulation trend of all annotated differential metabolites in the pathway, and −1 indicating a downregulation trend. The length of the line segment represents the absolute value of the DA Score. The size of the circles indicates the number of annotated differential metabolites in each pathway, with a larger circle indicating a higher number of metabolites. Circles located on the right side of the axis with longer line segments indicate a tendency towards upregulation in the overall expression of the pathway, while circles on the left side with longer line segments indicate a tendency towards downregulation

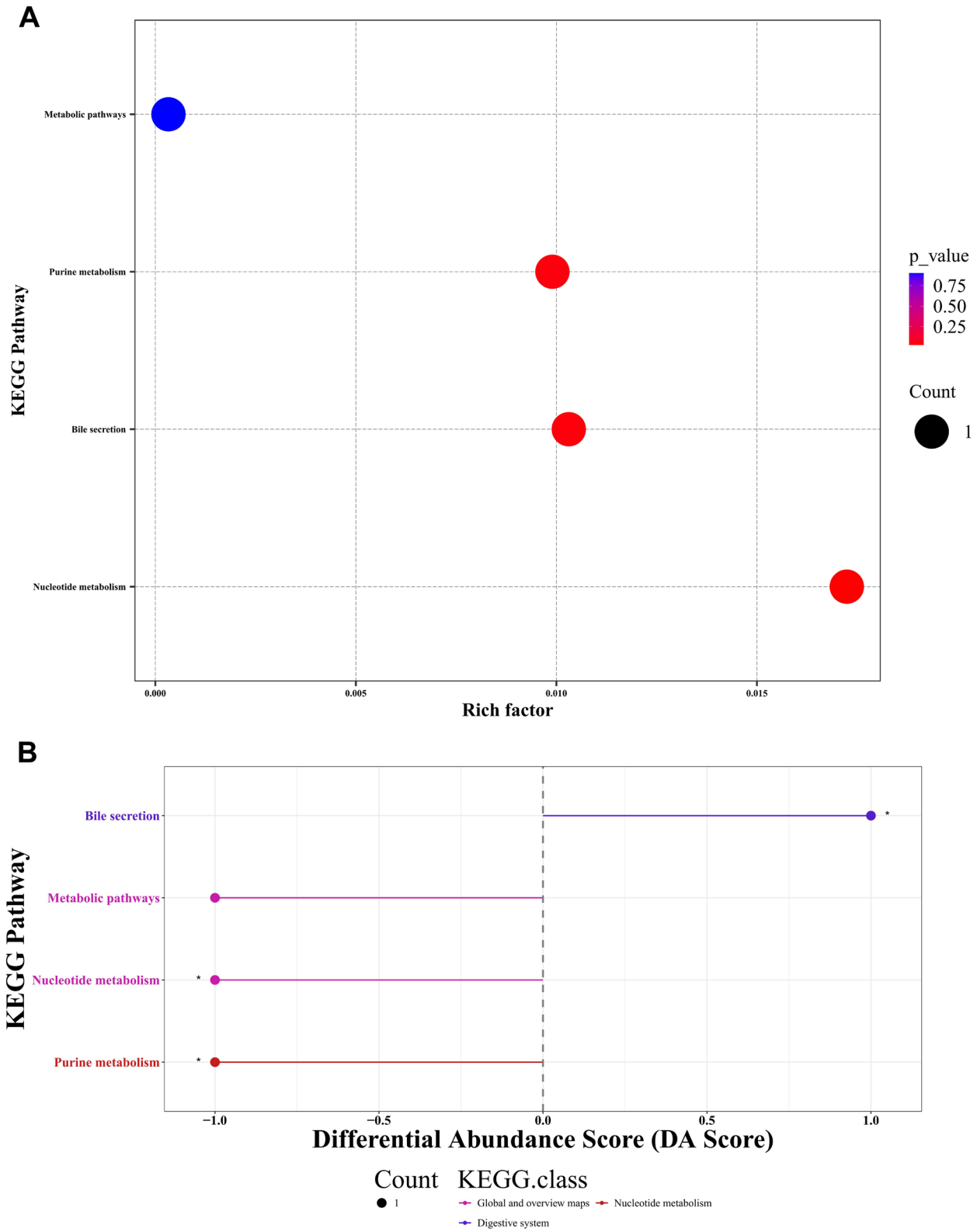

Fig. 12 (See legend on previous page.)

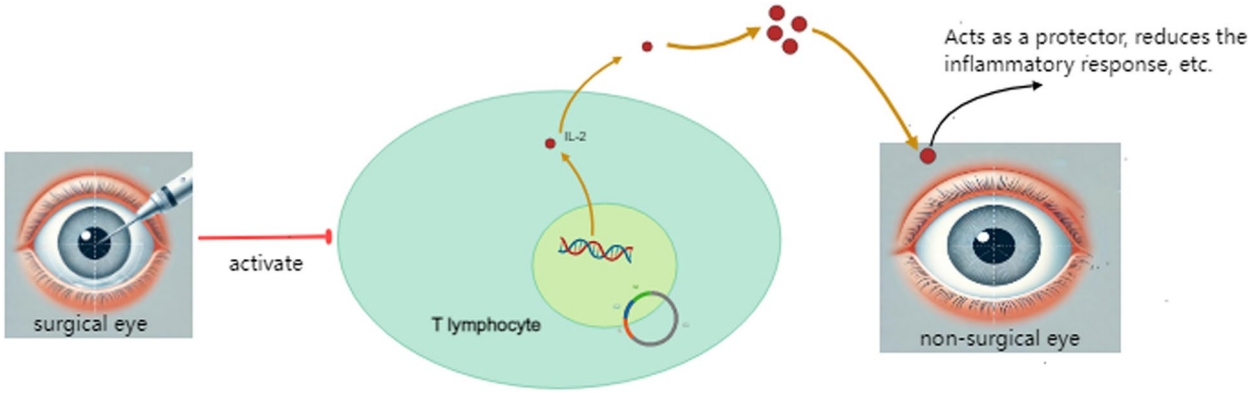

Fig. 13 The activation of T cell immunity and the release of IL-2 after the first eye surgery play a protective role in the contralateral eye

synthesis but is also associated with immune activation. Studies have shown that purine-based DNA can activate the immune system when transferred to the cytoplasm [67]. ATP, as a danger signal, activates immune cells, while adenosine inhibits inflammation [48, 66, 68, 69]. Our experimental results indicate that the downregulation of purine metabolism and upregulation of adenosine suggest that immune responses are suppressed, reducing immune activation and local damage in the non-operated eye. Based on the expression of metabolites involving nucleotide, carbohydrate, and lipid metabolism found in differential metabolites, we have reason to believe that there are metabolic disruptions and changes in the internal environment homeostasis in the non-operated eye following cataract phacoemulsification in the first eye. Although no changes were detected in selected cytokines in the non-operated eye, the differential metabolite expression suggests that the organism may be undergoing mild metabolic adjustments post-surgery, without triggering more complex biological responses such as cytokine alterations.

It is noteworthy that if patients have immune dysfunction or metabolic disorders such as diabetes, more complex physiological and biochemical processes may be further stimulated. This helps explain why changes in intraocular biomarkers are more easily detected in diabetic patients [28].

In pathway enrichment analysis, we did not observe enrichment of lipid metabolism pathways. However, differential metabolites included lipid substances, among which SM(d34:1) exhibited downregulation in second eyes. Sphingomyelin is an important lipid class that serves various functions in organisms, including cellular membrane structure and cell signaling [70]. In a mouse model of osteoarthritis (OA), it was found that SM(d34:1) is upregulated in the mouse metabolome and could serve as a potential pathological biomarker for OA [71]. In our experiment, however, SM(d34:1) was observed to be downregulated in the human body. This alteration in sphingomyelin homeostasis similarly indicates the impact of surgery on the aqueous humor homeostasis in the non-operated eye following surgery on one eye.

We found differential metabolites such as Carbobenzyloxy-L-norvalyl-L-norleucine and L-Serine methyl ester, which have not been definitively defined or reported in literature, but are likely associated with amino acid metabolism. They may be involved in amino acid synthesis, degradation pathways, or serve as precursors or metabolites in amino acid metabolism, playing crucial roles in protein synthesis, neurotransmitter synthesis, and other biological processes. Additionally, among the differential metabolites we detected, apart from those involving fundamental human metabolism like purine metabolism, nucleotide metabolism, and carbohydrate metabolism, N-alpha-methylhistamine showed increased expression. N-alpha-methylhistamine acts as an agonist for the H3 receptor, with activity approximately three times higher than histamine itself [72, 73]. However, when rigorously excluding systemic diseases and medication histories of patients, based on its chemical structure, N-alpha-methylhistamine is more likely to exist as a metabolic product of histamine [74]. Its high expression may indicate an abnormality in histamine metabolism in the AH of the non-operated eye. This further validates our hypothesis that surgery on one eye indeed affects the internal environment of the AH in the other eye. This effect likely plays a biological role in suppressing inflammatory responses and influencing immune regulation functions. Of course, this phenomenon and its effects require further extensive research and exploration.

In cytokine detection, we observed differences between clinical and animal experiments. We believe that although rabbits serve as an important model in human immune research [75], there are significant

immunological differences between rabbits and humans. Under the same stimuli, rabbits tend to exhibit a more intense immune response. In the clinical study, differential expression of adenine and purine metabolism was detected. A thorough literature review indicates that purinergic signaling interacts with other molecular pathways, forming a complex network that regulates various cellular processes, including proliferation, differentiation, and apoptosis [76]. These metabolic changes help maintain immune homeostasis in humans, whereas rabbits require additional metabolic pathways to counteract the impact of the first-eye surgery, leading to a more pronounced cytokine response. In our ongoing studies, we have also observed increased differential metabolites and metabolic pathway activation in the AH of the contralateral eye in rabbits. This may also explain why cataract patients with systemic diseases and immune dysfunction exhibit higher levels of inflammatory cytokines in the non-operated eye.

In the animal experiment, compared to the control group, the levels of IL-2 and IL-1β in the non-operated eye were elevated. Although no statistically significant differences were observed in the subgroup analysis at different time points after correcting for type I error, their mean values remained higher than those in the control group and showed a downward trend after two weeks postoperatively. Additionally, in the operated eye, cytokines such as IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and VEGF were significantly elevated within the first two weeks postoperatively. Although IFN-γ remained statistically significant at three weeks postoperatively, it also exhibited a declining trend after the second week. These findings suggest that AH microenvironment changes are most pronounced within the first two weeks postoperatively and tend to stabilize thereafter. Therefore, for patients with simple cataracts, performing the second-eye surgery at least two weeks after the first-eye surgery may help reduce immune stress responses. Furthermore, in the clinical study, we observed metabolic alterations in AH metabolites and metabolic pathways between the first and second eyes within two weeks postoperatively. This further supports the notion that performing second-eye surgery within this period may disrupt immune homeostasis. Based on cytokine and metabolic analyses, we recommend an interval of at least two weeks before proceeding with second-eye cataract surgery to optimize surgical outcomes and minimize the potential risk of postoperative immune responses.

Finally, although our results indicate inflammatory and metabolic changes in the aqueous humor of the contralateral eye, this study is only a preliminary exploration of postoperative changes, and further research is required to elucidate the precise physiological and biochemical mechanisms. In animal experiments, sampling time points were more precise, and specimens were typically collected during the early postoperative phase (e.g., 1 and 3 days), which facilitates capturing acute immune responses. In the clinical study, the variability in second-eye surgery timing made it difficult to collect samples at fixed early postoperative points (e.g., day 1, day 3), so a two-week time point was chosen instead. This difference in sampling timing may explain the discrepancies in inflammatory factor expression between animal experiments and the clinical study. Moreover, individual differences in human immune responses may contribute to variations in outcomes. A more detailed stratification of metabolite and cytokine changes at different postoperative time points in clinical trials could provide stronger clinical evidence. Additionally, the limited sample size in clinical trials and the relatively small number of rabbits in longitudinal comparisons (despite validation of initial sample quality) pose limitations. Future studies will expand sample sizes to enhance statistical power and further validate our findings. Nevertheless, this study provides a novel perspective on a common clinical issue.

Conclusion

Through this study, we conducted a comprehensive analysis of changes in biomarkers and metabolite profiles in patients undergoing bilateral cataract surgery. In our animal model, we observed a significant increase in the inflammatory cytokines IL-1β and IL-2 in the AH of the non-operated eye post-surgery, which may reflect both local and systemic inflammatory responses. Additionally, metabolomics analysis revealed differential expression of metabolites such as adenine, as well as alterations in purine and nucleotide metabolism in the non-operated eye following surgery. These findings suggest metabolic dysregulation and disruptions in internal homeostasis in the non-operated eye after unilateral surgery. Although no significant changes in cytokines were observed in human samples, alterations in metabolites point to subtle metabolic adjustments in the biological system. These findings indicate that unilateral cataract surgery may impact the stability of the intraocular environment in the contralateral eye, suggesting that potential metabolic changes in the non-operated eye, along with their clinical significance, should be considered in staged bilateral surgeries. This study provides important insights for optimizing postoperative management strategies, reducing complications, and determining appropriate timing for bilateral surgeries, warranting further investigation. Future studies with larger sample sizes and longer follow-up periods are needed to better understand the impact of these changes on patients undergoing bilateral surgery and to explore potential preventive and intervention measures.

Abbreviations

ARC	Age-related cataract
IOL	Intraocular lens
MCP-1	Monocyte Chemoattractant Protein-1
AH	Aqueous humor
CSF3	Colony Stimulating Factor 3
CCL2	C–C Motif Chemokine Ligand 2
MIP-1d	Macrophage Inflammatory Protein 1d
TNF-α	Tumor Necrosis Factor α
TGF-β2	Transforming Growth Factor β2
UHPLC-OE-MS	Ultra-high-performance liquid chromatography coupled with ultra-high-resolution mass spectrometry
LC/MS	Liquid Chromatography/Mass Spectrometry
cELISA	Concentrations sandwich enzyme-linked immunosorbent assay
IFN-γ	Interferon-γ
VEGF	Vascular endothelial growth factor
QC	Quality Control
OPLS-DA	Orthogonal projections to latent structures-discriminant analysis
VIP	Variable importance in the projection
KEGG	Kyoto Encyclopedia of Genes and Genomes
ROC	Receiver Operating Characteristic
DCs	Dendritic cells
SO	Sympathetic Ophthalmia
OA	Osteoarthritis

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12886-025-03961-9.

Supplementary Material 1: Appendix A. Supplementary data. The additional data of this article can be made available on a public website after publication and will be permanently preserved.

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Authors' contributions
LY and CTY proposed and designed the study, and wrote the main manuscript. LFY, ZSJ,LMB and GWJ collected clinical specimens. LY analyzed the data. CTY and LTX provided financial support and assisted with surgical procedures. LTX designed the experiment and reviewed the manuscript. All authors read and approved the final manuscript.

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Data availability
Data can be provided within the manuscript or as supplementary information upon request. Alternatively, the data will be made available in a public database immediately upon acceptance of the article.

Ethics approval and consent to participate
This study obtained approval from the Ethics Committee of Zunyi Medical University and adhered to the principles of the Helsinki Declaration. All patients signed written informed consent forms. The animal experiment was approved by the Welfare Ethics Committee (Application No.: ZMU21-2203–590), and the clinical study was approved by the Ethics Committee of the Second Affiliated Hospital of Zunyi Medical University (Approval No.: KYLL-2023–013).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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