An Approach to Predict Intraocular Diseases by Machine Learning Based on Vitreous Humor Immune Mediator Profile

Risa Sugawara,[1] Yoshihiko Usui,[1] Akira Saito,[2] Naoya Nezu,[1] Hiroyuki Komatsu,[1] Kinya Tsubota,[1] Masaki Asakage,[1] Naoyuki Yamakawa,[1] Yoshihiro Wakabayashi,[1] Masahiro Sugimoto,[3] Masahiko Kuroda,[4] and Hiroshi Goto[1]

[1]Department of Ophthalmology, Tokyo Medical University Hospital, Tokyo, Japan
[2]Department of AI Applied Quantitative Clinical Science, Tokyo Medical University, Tokyo, Japan
[3]Research and Development Center for Minimally Invasive Therapies, Institute of Medical Science, Tokyo Medical University, Tokyo, Japan
[4]Department of Molecular Pathology, Tokyo Medical University, Tokyo, Japan

Correspondence: Yoshihiko Usui, Department of Ophthalmology, Tokyo Medical University Hospital, 6-7-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 160-0023, Japan; usuyoshi@gmail.com.

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Purpose. This study aimed to elucidate whether machine learning algorithms applied to vitreous levels of immune mediators predict the diagnosis of 12 representative intraocular diseases, and identify immune mediators driving the predictive power of machine learning model.

Methods. Vitreous samples in 522 eyes diagnosed with 12 intraocular diseases were collected, and 28 immune mediators were measured using a cytometric bead array. The significance of each immune mediator was determined by employing five machine learning algorithms. Stratified k-fold cross-validation was performed to divide the dataset into training and test sets. The algorithms were assessed by analyzing precision, recall, accuracy, *F*-score, area under the receiver operating characteristics curve, area under the precision–recall curve, and feature importance.

Results. Of the five machine learning models, random forest attained the maximum accuracy in the classification of 12 intraocular diseases in a multi-class setting. The random forest prediction models for vitreoretinal lymphoma, endophthalmitis, uveal melanoma, rhegmatogenous retinal detachment, and acute retinal necrosis demonstrated superior classification accuracy, precision, and recall. The top three important immune mediators for predicting vitreoretinal lymphoma were IL-10, granzyme A, and IL-6; those for endophthalmitis were IL-6, G-CSF, and IL-8; and those for uveal melanoma were RANTES, IL-8 and bFGF.

Conclusions. The random forest algorithm effectively classified 28 immune mediators in the vitreous to accurately predict the diagnosis of vitreoretinal lymphoma, endophthalmitis, and uveal melanoma among 12 representative intraocular diseases. In summary, the results of this study enhance our understanding of potential new biomarkers that may contribute to elucidating the pathophysiology of intraocular diseases in the future.

Keywords: immune mediators, machine learning, biomarkers, intraocular diseases, vitreous

Inflammation is affected by numerous immunological mediators such as cytokines, chemokines, and growth factors and is associated with different intraocular diseases that may result in blindness. The causes of inflammation include ischemia, diabetes, autoimmunity, infections, and tumors, but may also be unidentified. Over the last decade, research has shown that the inflammatory mediators, which have multiple and overlapping effects, are present in the ocular fluids of patients with intraocular diseases and impact the development and progression of the diseases.[1–4] The mediators can trigger cascades of inflammatory events, drawing more immune mediators and inflammatory cells to the eye. Consequently, the recruitment, homing, activation, and differentiation of specific immune effects affect eyesight. The immune mediators are drawing increasing attention in the definition and investigation of the pathophysiological mechanisms underlying each disease phenotype, assuming that each phenotype is unique in terms of underlying immunological mechanism. Up-regulated or down-regulated immune mediators are also involved in the development and physical progression of various intraocular diseases, influencing their clinical characteristics that impact the diagnosis and treatment of these conditions.

We recently published the success of using random forest (RF) analysis of 28 immune mediators in the aqueous humor to accurately predict the diagnosis of vitreoretinal lymphoma, acute retinal necrosis, and endophthalmitis among 17 intraocular diseases.[5] However, it remains unclear whether intraocular diseases can be predicted by analyzing immune factors in vitreous humor using machine learning.

Although aqueous humor is easier and safer to sample than vitreous humor, immune mediator profiles in the vitreous may more accurately reflect the inflammatory state and pathological changes in intraocular diseases. Comprehensive analysis of immune mediators in the vitreous have been reported in several studies by our group and others, in vitreoretinal lymphoma and uveitis,[6,7] endophthalmitis,[8,9] uveal melanoma,[10,11] branch retinal vein occlusion,[12–14] and diabetic retinopathy,[15–20] as well as in other related research focused on various diseases including acute retinal necrosis.[21] However, there has been no comparative analysis of immune mediator levels in the vitreous across different intraocular diseases.

In the present study, we measured the levels of 28 immune mediators in vitreous samples obtained from patients diagnosed with 12 distinct intraocular diseases and developed machine learning models using stratified k-fold cross-validation to comprehensively analyze the vitreous immune mediators levels across the 12 diseases. Our objectives were to identify key immune mediators critical for discrimination of these diseases, assess their diagnostic significance, and use them to construct an innovative diagnostic tool within the machine learning framework. Additionally, we sought to explore how these vitreous immune mediators may be integrated with peripheral blood test data to enhance diagnostic accuracy.

Methods

Patients and Diagnosis

Patients with intraocular diseases were identified retrospectively from the medical records at Tokyo Medical University Hospital between 2007 and 2022. Vitreous samples were obtained from 522 eyes of 505 patients who were diagnosed with 12 different intraocular diseases (Table). These diseases were as follows: 42 eyes with acute retinal necrosis, 35 eyes with endophthalmitis, 45 eyes with sarcoidosis, 46 eyes with uveitis of unknown cause, 51 eyes with vitreoretinal lymphoma, 35 eyes with uveal melanoma, 85 eyes with rhegmatogenous retinal detachment, 43 eyes with proliferative diabetic retinopathy, 31 eyes with retinal vein occlusion, 40 eyes with idiopathic macular hole, 44 eyes with idiopathic epiretinal membrane, and 25 eyes with retinal hemangioma. Fifteen patients had bilateral involvement, comprising two patients with sarcoidosis, four patients with uveitis of unknown cause, and nine patients with vitreoretinal lymphoma. Two patients with acute retinal necrosis underwent reoperation. From these patients, 30 bilaterally affected eyes and four operated eyes were included in the analysis.

Table. Distribution of Vitreous and Blood Samples by 12 Intraocular Diseases

Disease	Vitreous Samples	Blood Samples
Acute retinal necrosis	42	42
Endophthalmitis	35	26
Sarcoidosis	45	34
Uveitis of unknown cause	46	43
Vitreoretinal lymphoma	51	42
Uveal melanoma	35	33
Rhegmatogenous retinal detachment	85	81
Proliferative diabetic retinopathy	43	35
Retinal vein occlusion	31	31
Idiopathic macular hole	40	36
Idiopathic epiretinal membrane	44	37
Retinal hemangioma	25	25
Total	522	465

Among the patients studied, 465 blood samples were collected, comprising 42 samples from acute retinal necrosis cases, 26 from endophthalmitis cases, 34 from sarcoidosis cases, 43 from uveitis cases of unknown cause, 42 from vitreoretinal lymphoma cases, 33 from uveal melanoma cases, 81 from rhegmatogenous retinal detachment cases, 35 from proliferative diabetic retinopathy cases, 31 from retinal vein occlusion cases, 36 from idiopathic macular hole cases, 37 from idiopathic epiretinal membrane cases, 25 eyes from retinal hemangioma cases. The diagnosis of vitreoretinal lymphoma was established based on clinical, morphologic, cytochemical, gene rearrangement, and immunologic features.

To compare cytokines in the vitreous and aqueous humor, we analyzed the following 10 diseases with matching diagnoses. The aqueous humor cytokine data were based on the following cases: 42 eyes with acute retinal necrosis, 22 eyes with endophthalmitis, 20 eyes with sarcoidosis, 33 eyes with uveitis of unknown cause, 30 eyes with vitreoretinal lymphoma, 26 eyes with uveal melanoma, 52 eyes with rhegmatogenous retinal detachment, 34 eyes with proliferative diabetic retinopathy, 21 eyes with retinal vein occlusion, 27 eyes with idiopathic macular hole, and 35 eyes with idiopathic epiretinal membrane. The cytokine data in aqueous humor were obtained from the data in our previous study.[5]

A diagnosis of acute retinal necrosis was based on the criteria published in 2015 for acute retinal necrosis in Japan[22] and the criteria of the American Uveitis Society.[23] DNA of herpes simplex virus, varicella-zoster virus or cytomegalovirus was detected using real-time polymerase chain reaction. The term endophthalmitis refers to intraocular bacterial and fungal infections of exogenous or endogenous origin. The diagnosis of ocular sarcoidosis was made according to the criteria revised in 2009 by the International Workshop On Ocular Sarcoidosis.[24] Uveitis of unknown cause was diagnosed when results of multiple clinical examinations and laboratory tests did not meet the criteria for any of the aforementioned disease categories. Vitreoretinal lymphoma was diagnosed according to current diagnostic criteria based on clinical characteristics, radiographic examination, blood tests, vitreous cytopathological examination, molecular genetic (such as gene rearrangement) analyses, and ratios of IL-6 to IL-10 in intraocular fluid. Uveal melanoma was diagnosed based on the histologic findings after enucleation. Rhegmatogenous retinal detachment was diagnosed during routine eye examination. Proliferative diabetic retinopathy was defined as diabetic retinopathy with obvious neovascularization with or without proliferative tissue. To examine the possible effect of the presence of blood in vitreous samples from eyes with proliferative diabetic retinopathy on immune mediator measurements, we compared the immune mediator profiles in the

absence and presence of vitreous hemorrhage. The levels of IL-10 and VEGF were lower in patients without hemorrhage than in those with hemorrhage. However, the number of patients without vitreous hemorrhage was very small (two cases) and the reliability of the test was low. Therefore, in this study, patients with proliferative diabetic retinopathy and vitreous hemorrhage were combined with those without hemorrhage and considered as diabetic retinopathy. Retinal vein occlusion was defined as a nonperfusion area of more than five disc diameters. Diagnoses of idiopathic macular hole, and idiopathic epiretinal membrane were determined based on typical ophthalmoscopic findings with additional specific examinations such as OCT and fluorescein angiography. retinal hemangioma. Idiopathic epiretinal membrane cases did not have a history of retinal tears or laser treatments. Except patients with proliferative diabetic retinopathy, none of the patients with other intraocular diseases had diabetes mellitus. This study was approved by the Ethics Committee of Tokyo Medical University Hospital, and written informed consent was obtained from all participants.

Samples

Vitreous humor samples (approximately 0.5 to 1.0 mL) were obtained from the mid-vitreous region at the start of standard three-port 25-gauge vitrectomies in cases other than melanoma. For melanoma cases, vitreous samples were collected by bisecting the enucleated eyeball. The undiluted samples were removed with a vitreous cutter before intraocular infusion and stored at −80°C until assay.

Peripheral blood samples were collected before vitrectomy. The following 26 hematological and biochemical tests were conducted: white blood cell count, red blood cell count, platelet count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, neutrophil, lymphocyte, monocyte, eosinophil, basophil, red cell distribution width, mean platelet volume, total protein, aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transpeptidase, total bilirubin, uric acid, urea nitrogen, creatinine, Na, Cl, K, and glucose.

Immune Mediators

Twenty-eight immune mediators were measured, comprising IL-1α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-17A, IFN-γ, tumor necrosis factor-α, interferon-γ-inducible protein (IP)-10, monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, MIP-1β, regulated on activation, expressed and secreted by normal T cells (RANTES), monokine induced by IFN-γ (Mig), VEGF, angiogenin, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor, basic fibroblast growth factor (bFGF), Fas ligand, granzyme A and granzyme B. Immune mediator concentrations were measured using a Cytometric Bead Array Flex immunoassay kit (BD Bioscience, Franklin Lakes, NJ, USA). Two-color flow cytometric analysis was performed using a FACSCalibur flow cytometer (BD Bioscience). Immunoassays were performed and analyzed according to the manufacturer's instructions.

Concentrations of immune mediators were calibrated against a standard curve for each cytokine. If concentrations in the raw data were below the detection limit, they were coded as 0 and included in the analysis. Because the numerical data in the raw dataset showed large variations, the data were converted to natural logarithm and used in all data analyses. All negative values after log transformation were set to 0.

Statistical Analysis

Machine learning models were implemented using Python (version 3.8). The primary libraries and packages used for the models include: Scikit-learn (version 0.24.2): This library was used for implementing the following machine learning algorithms: Radial Basis Function (RBF) Support Vector Machine (SVM): implemented using "SVC" with the "rbf" kernel; Linear SVM: implemented using "LinearSVC"; Decision Tree: implemented using "DecisionTreeClassifier"; Naive Bayes: implemented using "GaussianNB" for continuous data; Pandas (version 1.3.3): used for data manipulation and preprocessing; NumPy (version 1.21.2): used for numerical operations; and Matplotlib (version 3.4.3) and Seaborn (version 0.11.2): used for data visualization. The vitreous humor concentrations of 28 immune mediators in 12 intraocular diseases were visualized using a heatmap executed by Python.

Then we examined the possibility of predicting diseases from immune mediator profile by RF, Radial basis function SVM, Linear SVM, Decision Tree, and Naive Bayes. RF is a classifier that creates multiple decision trees, and the output is determined by the voting results of multiple trees.

We used 250 decision trees in this study. Radial basis function SVM is a kernel-based classifier that uses a radial basis function to map input data into a higher-dimensional space, allowing effective handling of non-linear relationships. Linear SVM is a type of SVM that finds the optimal hyperplane in a linear feature space to maximize the margin between different classes and is suitable for linearly separable data. Decision Tree is a model that uses a tree-like structure where each internal node represents a decision based on a feature, each branch represents the outcome of the decision, and each leaf node represents a class label. It is simple to interpret but prone to overfitting. Naive Bayes is a probabilistic classifier based on Bayes' theorem, assuming that features are conditionally independent given the class label. Despite its simplicity, it is effective for high-dimensional data.

To evaluate the generalizability of these models, k-fold cross-validation tests ($k = 5$) were performed. First, all data were split into five folds. One fold was selected as test data and the remaining four folds as training data, ensuring that different data sets were used for model training and testing each time. At each random state, a random number generator was varied between 1 to 100 to evaluate different new data 100 times, and then resampled. In other words, cross-validation was performed 500 times with different combinations. Finally, the score for each fold was summed and averaged to obtain the overall score. The average value of classification accuracy after 500 runs of stratified k-fold cross-validation was calculated. We evaluated the performance of the classification algorithms in terms of precision, recall, classification accuracy and F-score.

Next, we examined the degree of discrimination of one disease from the others. The model was reconstructed after transforming the target disease into a binary variable of 1 and the remaining diseases into a binary variable of 0. The performance of the model was evaluated based on the area under the curve (AUC) of the receiver operating character-

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istic (ROC) and the precision-recall (PR) curve. The AUC-ROC provides a measure of the model's ability to distinguish between classes across different threshold settings, while the PR curve focuses on the trade-off between precision and recall, which is particularly useful in cases of imbalanced datasets. The importance of each immune mediator is expressed as feature importance, which is an indicator of how much each feature contributes to the performance of the model during the process of training the model. All RF models were implemented in Python 3.8.3 (Python Software Foundation, Wilmington, DE, USA). Finally, to investigate the usefulness of combining vitreous immune mediator concentrations with peripheral blood test data, we applied these combined data to RF algorithm.

Results

Hierarchical Clustering of 28 Immune Mediators in 12 Intraocular Diseases

We conducted hierarchical clustering of 28 immune mediators across 12 intraocular diseases to investigate the differences between the diseases, as shown in Figure 1. Hierarchical clustering classified the 12 diseases into four distinct groups based on the immune mediators: (1) idiopathic epiretinal membrane and idiopathic macular hole; (2) proliferative diabetic retinopathy, retinal hemangioma, rhegmatogenous retinal detachment and retinal vein occlusion; (3) vitreoretinal lymphoma, uveal melanoma, uveitis of unknown cause and sarcoidosis; and (4) acute retinal necrosis and endophthalmitis.

Notably, levels of cytokines such as IL-8, IL-6, Mig, and IP-10 were remarkably lower in idiopathic epiretinal membrane and idiopathic macular hole than in the other diseases, whereas IL-8 and IL-6 were remarkably higher in acute retinal necrosis and endophthalmitis compared with other diseases. These results suggest differential immune mediator profiles in various intraocular diseases.

Machine Learning Using Random Forest to Predict 12 Intraocular Diseases

To select the most appropriate classifier for predicting 12 intraocular diseases using 28 immune mediators, we assessed five machine learning algorithms: RF, linear SVM, radial basis function SVM, decision tree, and Naïve Bayes Classifier. As shown in Figure 2, the predictive accuracies expressed in F scores were 60.2 ± 4.2 for RF, 53.6 ± 4.8 for linear SVM, 56.1 ± 4.1 for radial basis function SVM, 46.3 ± 5.0 for Decision Tree, and 36.9 ± 5.4 for Naïve Bayes Classifier. RF demonstrated significantly higher accuracy compared to the other four algorithms. Consequently, further analyses in this study were conducted using RF. Inclusion of both eyes in some patients due to bilateral involvement or reoperation in the analysis did not affect the correct response rate or order.

Next, we performed machine learning using RF to determine which immune mediators are important for predicting individual intraocular diseases. Figure 3A shows the average confusion matrix obtained with fivefold cross-validation. The vertical columns represent the actual classes, referring to the true category that the machine learning model is trying to predict. On the other hand, the horizontal rows represent the predicted classes, or the results of prediction made by the machine learning models. The average confusion matrix constructed indicated that the classifier was able to distinguish rhegmatogenous retinal detachment with high probability and to distinguish vitreoretinal lymphoma, endophthalmitis and uveal melanoma partially from the other diseases. However, it was more difficult to separate the other eight intraocular diseases (Fig. 3B). Nevertheless, the number of rhegmatogenous retinal detachment cases was

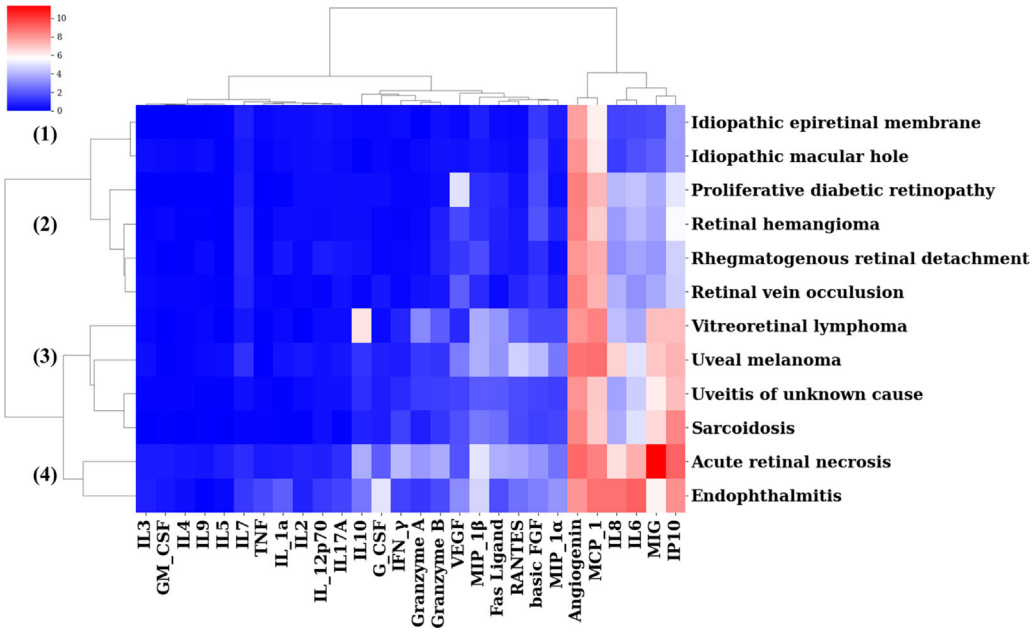

Figure 1. Heatmap of concentrations of 28 immune mediators in 522 vitreous samples collected from eyes diagnosed with 12 intraocular diseases. Data were log transformed, and all negative values after log transformation were set to 0. The averaged values for each disease are color coded in a *blue-white-red* (low to high) scale. Clustering was conducted using Pearson correlation. Prominent clusters are labeled (1) to (4).

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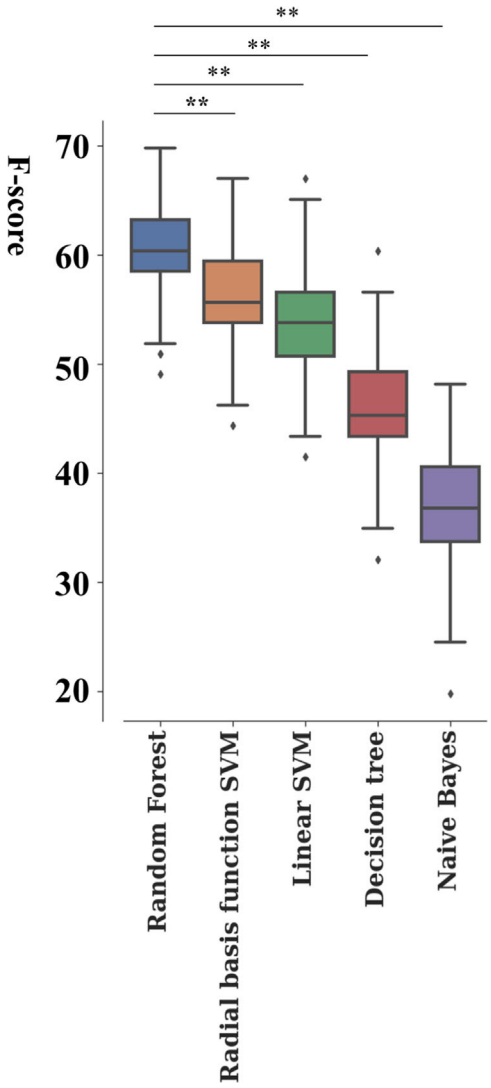


Figure 2. Boxplot comparing the accuracy of five machine learning algorithms using vitreous concentrations of 28 immune mediators to predict 12 intraocular diseases. Each *F*-score was obtained from 100 independent iterations of stratified fivefold cross-validation. The accuracy is significantly higher in RF compared with the other four algorithms. NB, naïve Bayes classifier; RBF, radial basis function. ** $P < 0.001$ (Mann–Whitney U test).

almost double the number of cases in the other diseases, and the result may have been influenced by biases.

Recall values up to the third prediction were calculated to investigate how many serious diseases could be candidates. Recall values as a correct answer up to the third prediction were calculated for each disease (Fig. 3B). Recall values up to the third prediction for the top five intraocular diseases were as follows: 95.2 for vitreoretinal lymphoma, 94.2 for rhegmatogenous retinal detachment, 91.6 for endophthalmitis, 90.4 for uveal melanoma, and 90.4 for idiopathic epiretinal membrane. Next, we calculated *F*-score, which is the harmonic mean to balance recall and prediction (Fig. 4). Overall mean accuracy for the 12 intraocular diseases was 60.3 ± 4.2. The F-scores for predicting the top five intraocular diseases were 89.5 ± 7.1 for vitreoretinal lymphoma, 81.7± 12.4 for endophthalmitis, 75.8 ± 11.6 for uveal melanoma, 72.9 ± 8 for rhegmatogenous retinal detachment, and 67.8 ± 11.9 for acute retinal necrosis.

Machine Learning Using Immune Mediators and Peripheral Blood Test Data to Predict 12 Intraocular Diseases

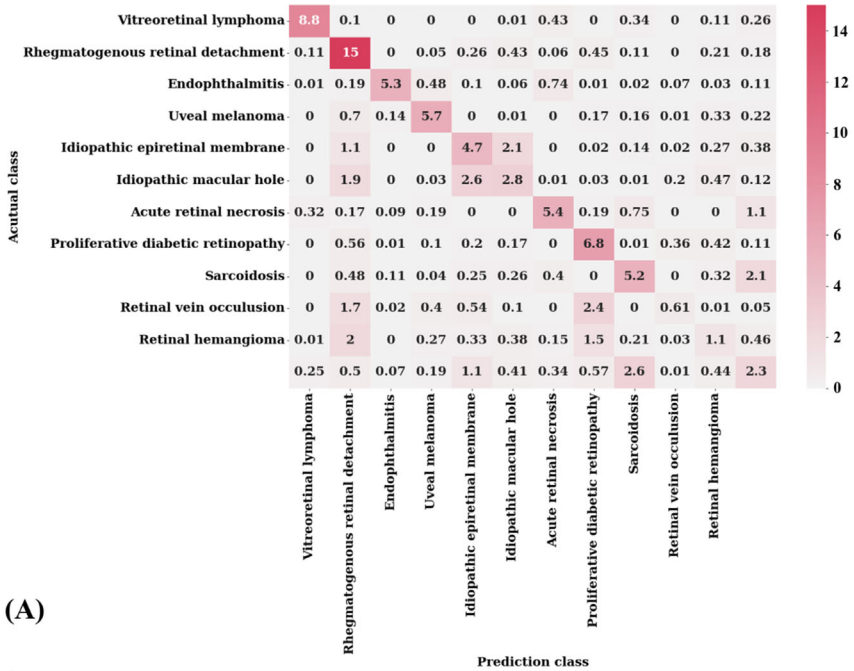
Finally, to examine the usefulness of using 28 immune mediators in vitreous combined with 26 blood test data in predicting 12 intraocular diseases, we analyzed the performance of prediction by machine learning using RF (Fig. 5). Overall mean accuracy was 60.9 ± 4.2, almost the same as that when using immune mediators only. There were no significant differences in *F*-scores for individual diseases between using immune mediators only (Fig. 4B) and using immune mediators combined with blood test results. The *F*-scores of using immune mediators and blood test data for predicting the top five intraocular diseases were 87.7 ± 11.3 for vitreoretinal lymphoma, 79.6 ± 15 for endophthalmitis, 76.4 ± 12.4 for uveal melanoma, 73 ± 7.4 for rhegmatogenous retinal detachment, and 69.1 ± 12.4 for acute retinal necrosis, also similar to those when using immune mediators alone. We also analyzed prediction of 12 intraocular diseases using the 26 blood test data only (Fig. 6). Overall mean accuracy was 28 ± 3.7. Accuracy based on immune mediators was significantly higher than accuracy based on blood tests. The *F*-scores of using blood tests alone for predicting the top five intraocular diseases were 43.7 ± 7 for rhegmatogenous retinal detachment, 38.7 ± 13.6 for vitreoretinal lymphoma, 38.2 ± 16.8 for retinal hemangioma, 33.4 ±15.2 for proliferative diabetic retinopathy, and 18.4 ± 16 for retinal vein occlusion. The prediction performance of using peripheral blood test data was apparently lower compared to using immune mediators combined with blood test results or immune mediators only.

Predictive Performance and Importance of 28 Immune Mediators to Differentiate one Disease From Others

We further evaluated the predictive performance of 28 immune mediators using ROC and PR to determine how well they differentiate one disease from the others (Fig. 7). The best performance, with an AUC-ROC of 0.985 ± 0.005, was observed in vitreoretinal lymphoma, followed by rhegmatogenous retinal detachment and proliferative diabetic retinopathy both having AUC-ROC of 0.955 ± 0.005, and then acute retinal necrosis with an AUC of 0.935 ± 0.005. The AUC-PR for these diseases were 0.935 ± 0.015 for vitreoretinal lymphoma, 0.825 ± 0.45 for endophthalmitis, and 0.82 ± 0.02 for rhegmatogenous retinal detachment.

We then assessed the most important immune mediators for the top five diseases with the highest F-score (Fig. 8). IL-10 was the most crucial mediator for vitreoretinal lymphoma, followed by granzyme A, IL-6, and IP-10. The primary predictors for endophthalmitis were IL-6, G-CSF, and IL-8. For uveal melanoma, RANTES, IL-8, and bFGF were the primary predictors. For rhegmatogenous retinal detachment, MCP-1 was the key predictor, followed by IP-10 and Mig. For acute retinal necrosis, IFN-γ was the most important, followed by Mig and granzyme A.

Figure 9 demonstrates the most important features (IL-10, MCP-1, VEGF, IFN-γ, and IL-6) for the top five diseases with the highest *F*-scores. Vitreous IL-10 level was



(A)

Prediction class

	First Prediction (disease)	Total % up to first prediction	Second Prediction (disease)	Total % up to second prediction	Third Prediction (disease)	Total % up to third prediction
Vitreoretinal lymphoma	Vitreoretinal lymphoma	87.6	Acute retinal necrosis	91.8	Sarcoidosis	95.2
Rhegmatogenous retinal detachment	Rhegmatogenous retinal detachment	89.0	Proliferative diabetic retinopathy	91.6	Idiopathic macular hole	94.2
Endophthalmitis	Endophthalmitis	74.4	Acute retinal necrosis	84.8	Uveal melanoma	91.6
Uveal melanoma	Uveal melanoma	76.6	Rhegmatogenous retinal detachment	86.0	Retinal hemangioma	90.4
Idiopathic epiretinal membrane	Idiopathic epiretinal membrane	53.8	Idiopathic macular hole	77.9	Rhegmatogenous retinal detachment	90.4
Idiopathic macular hole	Idiopathic macular hole	34.3	Idiopathic epiretinal membrane	66.1	Rhegmatogenous retinal detachment	89.2
Acute retinal necrosis	Acute retinal necrosis	65.8	Uveitis of unknown cause	74.9	Sarcoidosis	88.3
Proliferative diabetic retinopathy	Proliferative diabetic retinopathy	77.8	Rhegmatogenous retinal detachment	82.6	Retinal vein occulusion	87.4
Sarcoidosis	Sarcoidosis	56.8	Uveitis of unknown cause	79.7	Rhegmatogenous retinal detachment	85.0
Retinal vein occulusion	Retinal vein occulusion	10.5	Proliferative diabetic retinopathy	51.6	Rhegmatogenous retinal detachment	80.7
Retinal hemangioma	Retinal hemangioma	17.1	Rhegmatogenous retinal detachment	40.3	Proliferative diabetic retinopathy	57.4
Uveitis of unknown cause	Uveitis of unknown cause	12.5	Sarcoidosis	38.7	Idiopathic epiretinal membrane	51.2

(B)

Figure 3. Prediction of 12 intraocular diseases using RF based on 28 vitreous immune mediators. Data were obtained from 100 independent iterations of stratified fivefold cross-validation. Finally, the scores for each fold were summed and averaged. **(A)** Average confusion matrix constructed to examine whether the predicted classes match the actual classes in the intraocular disease cohort. The rows of the matrix represent the actual classes, and the columns represent the predicted classes. Each cell shows the number of cases where the actual class (row) is predicted as the class in the column. **(B)** Diagram showing recall results from the first to the third prediction in predicting 12 intraocular diseases by random forest modeling. Cumulative percentage of prediction is given next to each predicted disease.

significantly higher in vitreoretinal lymphoma than in all the other 11 intraocular diseases. IL-6 level was significantly higher in endophthalmitis. RANTES level was significantly higher in uveal melanoma than in all other diseases. IFN-γ was significantly elevated in acute retinal necrosis compared to the other diseases. However, MCP-1 level did not differ significantly between rhegmatogenous retinal detachment and the other diseases. These results suggested that some immune mediators in vitreous humor were closely associated with the pathogenesis of specific diseases. Our findings indicate that predicting the diagnosis of intraocular diseases may be feasible using machine learning to analyze a panel of immune mediators.

Discussion

Many immune mediators have been reported to be either upregulated or downregulated in certain intraocular diseases. Leukocytes infiltrating the eye and ocular resident cells such as retinal neurons, pigmented epithelial cells and glial cells can produce various kinds of immune mediators.[25–27] Therefore the results reported in the present article most likely correspond to a combination of immune mediators produced by immune cells and ocular resident cells, possibly interacting with each other. To the best of our knowledge, this is the first study that applied several machine learning algorithms to predict multiple intraocular diseases by analyzing immune mediators in vitreous samples.

Identification of the most important candidate immune mediators for predicting diagnosis of intraocular disorders is currently an important challenge. Therefore we aimed to investigate which of the 28 immune mediators are important for the prediction of individual intraocular diseases. We believe that machine learning is a powerful technology for discovering robust candidate markers for diagnosis and treatment.

In this study, we used RF, linear SVM, RBF SVM, Decision Tree, and naïve Bayes classifiers to construct models aiming at predicting the diagnosis of 12 intraocular diseases using 28 immune mediators in the vitreous. We compared all the models and found that RF demonstrated the best performance and achieved the highest classification accuracy, findings that agreed with those reported previously

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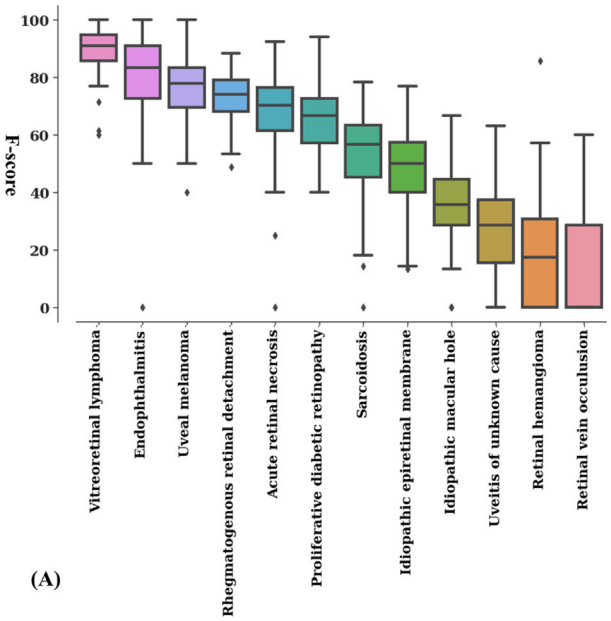
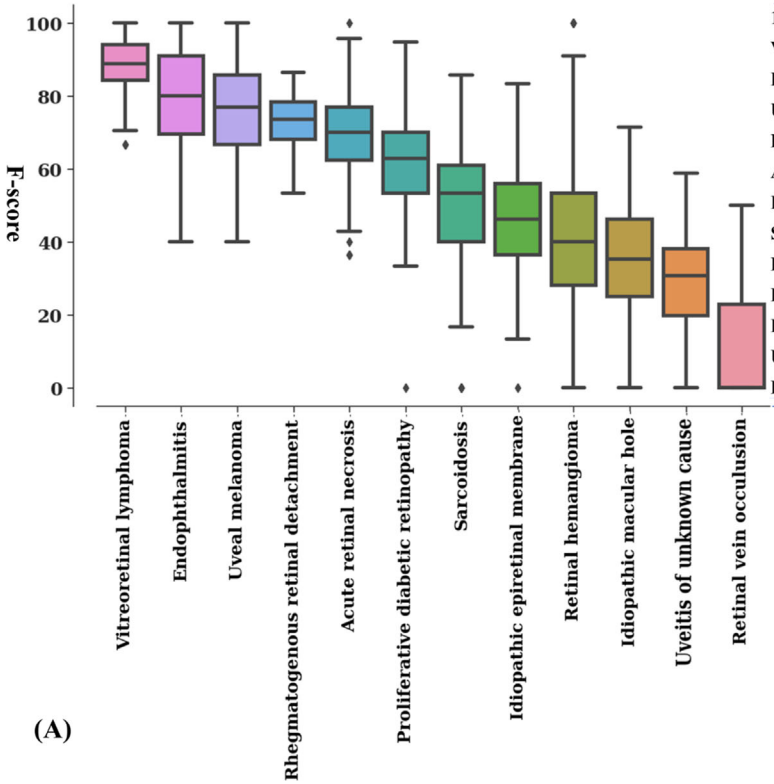


	Accuracy	F-score
12 classes	60.3 ± 4.2	
Vitreoretinal lymphoma		89.5 ± 7.1
Endophthalmitis		81.7 ± 12.4
Uveal melanoma		75.8 ± 11.6
Rhegmatogenous retinal detachment		72.9 ± 8
Acute retinal necrosis		67.8 ± 11.9
Proliferative diabetic retinopathy		64.6 ± 11
Sarcoidosis		54.5 ± 13.1
Idiopathic epiretinal membrane		48.7 ± 14
Idiopathic macular hole		37.4 ± 12.1
Uveitis of unknown cause		27.5 ± 14.2
Retinal hemangioma		18.6 ± 17
Retinal vein occulusion		15 ± 18

(B)

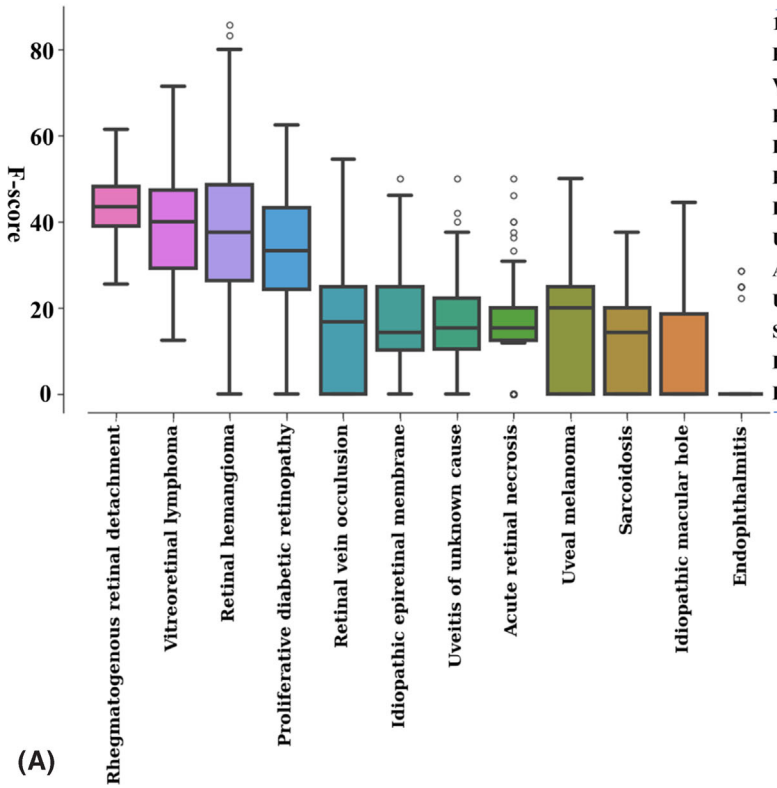
Figure 4. *F*-scores using random forest based on 28 vitreous immune mediators to predict 12 intraocular diseases. Each *F*-score was obtained from 100 independent iterations of stratified fivefold cross-validation. **(A)** Boxplot of *F*-scores for the 12 intraocular diseases. **(B)** Chart of overall accuracy for 12 classes and *F*-scores for 12 individual diseases.



	Accuracy	F-score
12 classes	60.9 ± 4.2	
Vitreoretinal lymphoma		87.7 ± 11.3
Endophthalmitis		79.6 ± 15
Uveal melanoma		76.4 ± 12.4
Rhegmatogenous retinal detachment		73 ± 7.4
Acute retinal necrosis		69.1 ± 12.4
Proliferative diabetic retinopathy		61.7 ± 13.3
Sarcoidosis		49.3 ± 17
Idiopathic epiretinal membrane		45.6 ± 15.3
Retinal hemangioma		41.1 ± 22.8
Idiopathic macular hole		34.1 ± 14.8
Uveitis of unknown cause		29.6 ± 13.1
Retinal vein occulusion		11.8 ± 15.1

(B)

Figure 5. Prediction of 12 intraocular diseases using random forest based on 28 vitreous immune mediators combined with 26 blood test data. Data were obtained from 100 independent iterations of stratified fivefold cross-validation. Finally, the scores for each fold were summed and averaged. **(A)** Boxplot of *F*-scores for the 12 intraocular diseases. **(B)** Chart of overall accuracy for 12 classes, and *F*-scores for 12 individual diseases.



	Accuracy	F-score
12 classes	28 ± 3.7	
Rhegmatogenous retinal detachment		43.7 ± 7
Vitreoretinal lymphoma		38.7 ± 13.6
Retinal hemangioma		38.2 ± 16.8
Proliferative diabetic retinopathy		33.4 ± 15.2
Retinal vein occulusion		18.4 ± 16
Idiopathic epiretinal membrane		16.1 ± 11.7
Uveitis of unknown cause		16 ± 11
Acute retinal necrosis		15.6 ± 11.1
Uveal melanoma		15.5 ± 15
Sarcoidosis		11.8 ± 11.7
Idiopathic macular hole		8.9 ± 11.9
Endophthalmitis		1.8 ± 6.6

(B)

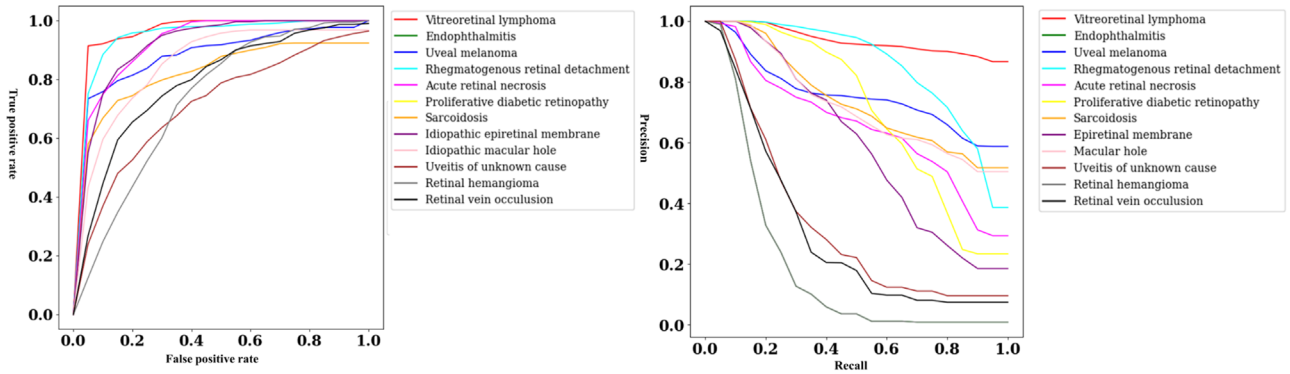
Figure 6. Prediction of 12 intraocular diseases using random forest based on 26 blood test data only. Data were obtained from 100 independent iterations of stratified fivefold cross-validation. Finally, the scores for each fold were summed and averaged. **(A)** Boxplot of *F*-scores for the 12 intraocular diseases. **(B)** Chart of overall accuracy for 12 classes, and *F*-scores for 12 individual diseases.

for aqueous humor immune mediators.[5] Although both eyes of 15 patients with bilateral involvement and both operated eyes of two patients with reoperation were included in the analysis, the results remained the same even when only patients with one affected eye were analyzed. It is possible that the random forest properties well match the nature of the cytokine data. RF uses a large number of decision trees, each trained on a random subset of features, to successfully capture important features while avoiding over-fitting. Other machine learning models are linear models that cannot capture the relationship between nonlinear features (SVM), have poor performance when parameters are not properly adjusted (radial basis function SVM), are single models that are prone to over-fitting (decision tree), or have poor performance when features are correlated (Naïve Bayes Classifier). Consequently, RF is the most suitable model for the purpose of the present study. The results of classifying 12 intraocular diseases in the present study provide the first evidence that machine learning algorithms applied to vitreous immune mediators can be used to discriminate specific intraocular diseases including vitreoretinal lymphoma, endophthalmitis, and uveal melanoma.

Considering that endophthalmitis, acute retinal necrosis, and vitreoretinal lymphoma are important vision-threatening intraocular diseases that share many common clinical features with other milder diseases, misdiagnosis may occur, causing delay in diagnosis and treatment with increased risk of blindness. The diagnosis of endogenous endophthalmitis was initially missed in nearly one-half of the cases,[28] and missed diagnosis of uveal melanoma was reported in 20% of the cases.[29] In addition, vitreoretinal lymphoma and uveal melanoma tend to be misdiagnosed.[30] However, in this study, including these difficult-to-diagnose conditions, the accuracy of identifying endophthalmitis, vitreoretinal lymphoma, and uveal melanoma exceeded 90% by the third prediction. This study suggests that integration of clinical data with immune mediators in machine learning may refine the diagnostic models. Incorporating demographic information, clinical features such as fundus characteristics, and immune mediators in the vitreous could enhance and fine-tune diagnostic models in the future.

On the other hand, we conducted additional analysis using RF models by inputting peripheral blood test data at the time of vitrectomy with or without vitreous immune mediator data, and also analysis of blood test data only. The analyses showed that combining immune mediator data with peripheral blood data did not improve the model so much compared with immune mediator data alone. Adding peripheral blood test results yielded similar accuracy rates as those obtained using only vitreous data, indicating that the inclusion of peripheral blood data did not contribute to substantial improvement. However, the key blood parameters that could better reflect the disease state, such as HbA1c and CRP, were not measured. This omission could also account for the lack of improvement in predictive accuracy.

In this study, several cytokines were shown to be useful in differentiating intraocular diseases. Host immune response to infection has been suggested to be an important factor in the pathogenesis of endophthalmitis. Hence, immune mediators produced as a result of host immune response, such as



(A) **(B)**

	Area under the Receiver Operating Characteristic Curve	95% Confidence Interval	Area under the Precision-Recall Curve	95% Confidence Interval
Vitreoretinal lymphoma	0.985	0.98-0.99	0.935	0.92-0.95
Rhegmatogenous retinal detachment	0.955	0.95-0.96	0.82	0.80-0.84
Proliferative diabetic retinopathy	0.955	0.95-0.96	0.675	0.63-0.70
Acute retinal necrosis	0.935	0.93-0.94	0.72	0.69-0.75
Endophthalmitis	0.93	0.91-0.95	0.825	0.78-0.87
Idiopathic epiretinal membrane	0.92	0.91-0.93	0.545	0.50-0.59
Uveal melanoma	0.9	0.88-0.92	0.75	0.71-0.79
Idiopathic macular hole	0.875	0.86-0.89	0.45	0.41-0.49
Sarcoidosis	0.855	0.83-0.88	0.58	0.53-0.63
Retinal vein occulusion	0.795	0.77-0.82	0.25	0.22-0.28
Retinal hemangioma	0.735	0.71-0.76	0.17	0.15-0.19
Uveitis of unknown cause	0.725	0.70-0.75	0.31	0.28-0.34

(C)

Figure 7. Performance of random forest to predict 12 intraocular diseases and discriminate each disease from the others, based on 28 immune mediators in vitreous. Data were obtained from 100 independent iterations of stratified fivefold cross-validation. **(A)** Line graphs showing average ROC curves for 12 intraocular diseases. **(B)** Line graphs showing average PR curves for 12 intraocular diseases. **(C)** Chart of AUC-ROC and AUC-PR for 12 intraocular diseases.

IL-6 as shown in the present study, may serve as a potential biomarker predicting the clinical outcome.

Vitreous IL-10, granzyme A and IL-6 were the most important immune mediators for predicting a diagnosis of vitreoretinal lymphoma. IL-10 and IL-6 are well known diagnostic biomarkers of vitreoretinal lymphoma.[7] However, IL-10/IL-6 < 1 was observed in approximately 10% of patients diagnosed with vitreoretinal lymphoma. Hence, ophthalmologists should pay special attention when IL-10/IL-6 < 1 in the differential diagnosis of vitreoretinal lymphoma from other inflammatory conditions. As anticipated, the increases of IL-10 and IL-6 in the vitreous significantly influenced the accuracy of RF models in discriminating vitreoretinal lymphoma. Surprisingly, granzyme A was the second most important mediator impacting accuracy. Using IL-10 and IL-6 in conjunction with granzyme A, as identified through machine learning algorithms, may enhance the likelihood of accurately diagnosing vitreoretinal lymphoma.

In this study, vitreous MCP-1, IP-10, and MIG (top three highest ranked prediction accuracy values) were the most important immune mediators for predicting rhegmatogenous retinal detachment. Although the level of MCP-1increases significantly in intraocular fluids of eyes with many intraocular diseases[5,6,10,11,13,21,31,32] and this chemokine was the top ranked predictor for rhegmatogenous retinal detachment in this study, MCP-1 appears to be a general indicator of ocular inflammation and lack specificity for distinct intraocular diseases. An animal study showed that retinal detachment caused augmented MCP-1 expression in Müller glia and increased $CD11b^+$ macrophages/microglia in the detached retina, mediating photoreceptor apoptosis.[32] Therefore MCP-1 inhibition may be a novel therapeutic approach for photoreceptor death in cases of complicated retinal detachment. On the other hand, previous studies have reported increased levels of IP-10 and MIG in the intraocular fluid of patients with rhegmatogenous retinal detachment.[33-35] IP-10 is a proinflammatory chemoattractant for monocytes and macrophages and functions as an anti-angiogenic and antifibrotic agent.[36] Therefore IP-10 may attract leukocytes to the inflamed area of retinal detachment. MIG regulates the migration of Th1 lymphocyte.[37] And, the presence of T lymphocytes has been reported vitreous and subretinal fluid,[38] as well as epiretinal membranes[39,40] in proliferative vitreoretinopathy, suggesting that MIG may be involved in the migration of Th1 lymphocytes.

RANTES, also known as chemokine ligand 5, was investigated in previous studies of uveal melanoma,[41–43] and the reported finding of increased RANTES was also observed in the present study. Nagarkatti-Gude et al.[41] reported significant increases of not only RANTES, but also IL-6, IL-8, IP-10, MCP-1, MIP-1α, MIP-1β, and TNF-α in the vitreous of eyes with uveal melanoma. It was reported that co-expression of RANTES with others chemokines results in significantly better T-cell response against uveal tumor cells[44,45] Uveal melanoma is a target for immunotherapy,[45] and activation of angiogenic and inflammatory pathways has been demon-

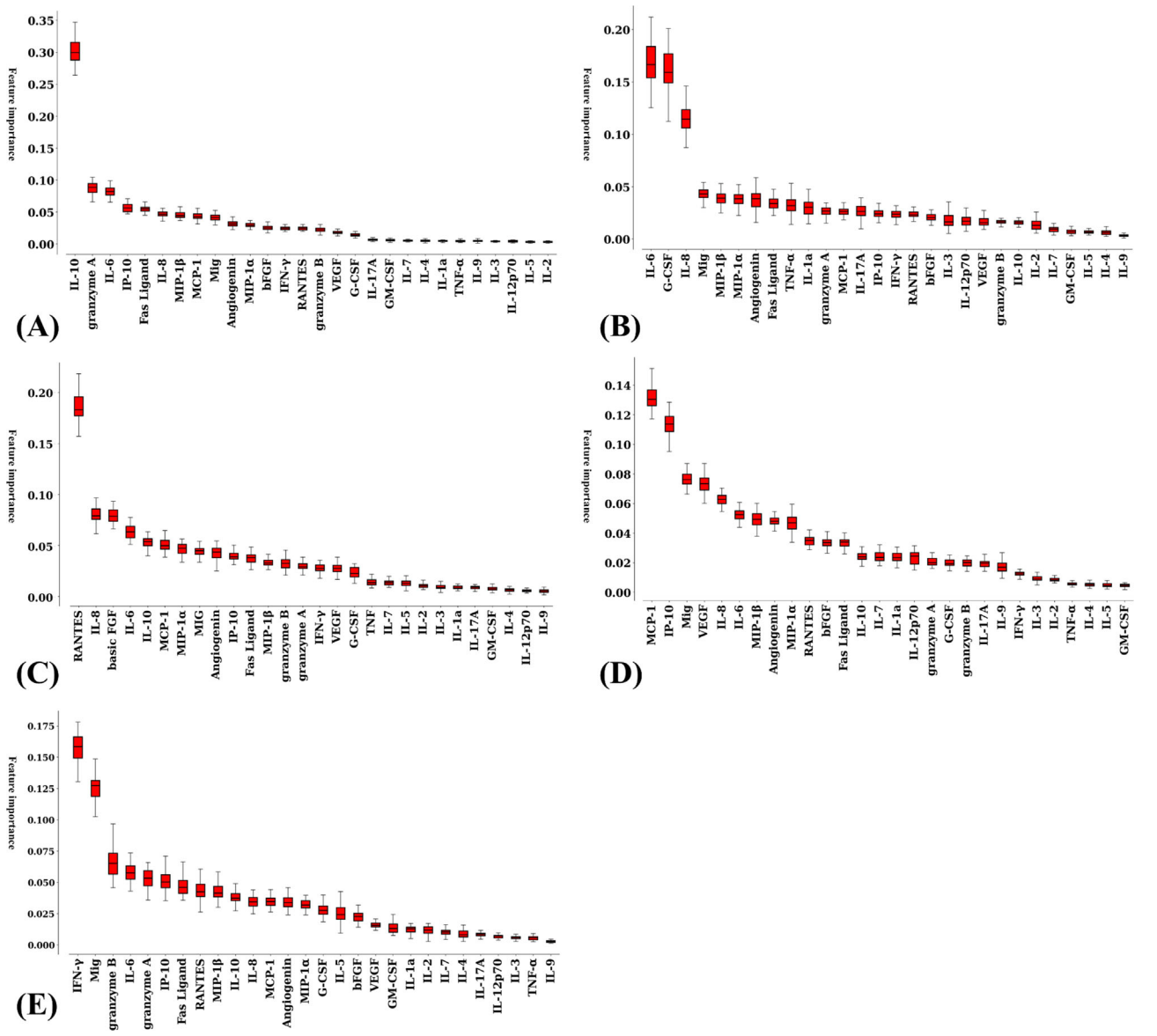


Figure 8. Boxplots showing the relative importance of 28 immune mediators for discriminating each disease from the others using random forest. The boxplots for the top five intraocular diseases with the highest *F*-score are shown: **(A)** vitreoretinal lymphoma, **(B)** endophthalmitis, **(C)** uveal melanoma, **(D)** rhegmatogenous retinal detachment, and **(E)** acute retinal necrosis. The importance of each immune mediator was computed as the feature importance. The data were obtained after 100 independent iterations of stratified fivefold cross-validation.

strated in uveal melanoma.[46] Therefore RANTES is most likely associated with T cells infiltrating uveal melanoma. However, previous studies were constrained by the nonstratified analytical approach. Interestingly, using machine learning algorithms, increased vitreous concentration of RANTES was the most important feature among 28 immune mediators for predicting uveal melanoma. Therefore, although many immune mediators increase significantly in uveal melanoma, RANTES may be the most useful therapeutic target.

Referring to our previous study, the top three predicted diseases when using aqueous humor were vitreoretinal lymphoma, acute retinal necrosis, and endophthalmitis.[5] Meanwhile, the top three predicted diseases when using vitreous samples in the present study were vitreoretinal lymphoma, endophthalmitis, and uveal melanoma, with acute retinal necrosis ranking the fifth. For vitreoretinal lymphoma, important immune mediators in aqueous humor were IL-10, angiogenin, and IP-10; while key predicting factors in the vitreous were IL-10, granzyme A, and IL-6. For endophthalmitis, the most important immune predictors in both aqueous humor and vitreous were IL-6, G-CSF, and IL-8. For uveal melanoma, the crucial immune predictors in the vitreous were RANTES and IL-8. In some diseases such as vitreoretinal lymphoma and endophthalmitis, the predictors in the vitreous showed high accuracy in prediction.

When the cytokine levels between aqueous humor and vitreous were compared using unpaired *t*-test, significant differences were found for the following cytokines: IL-10, granzyme A, and angiogenin for vitreoretinal lymphoma; RANTES, IL-8, and basic FGF for uveal melanoma; IL-6 for endophthalmitis. The largest difference was observed in uveal melanoma, where vitreous cytokine samples were

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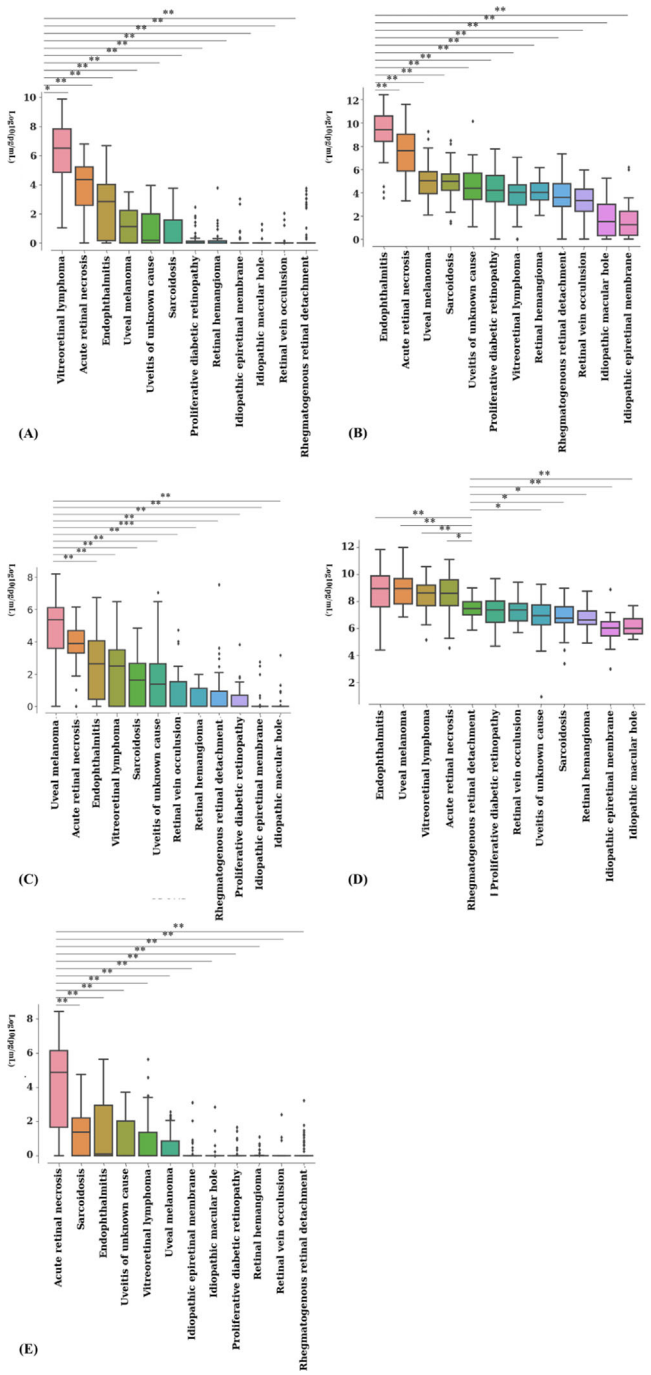

Figure 9. Boxplots illustrating the most important features for the top five intraocular diseases with the highest F-score: **(A)** IL-10 for vitreoretinal lymphoma, **(B)** IL-6 for endophthalmitis, **(C)** RANTES for uveal melanoma, **(D)** MCP-1 for rhegmatogenous retinal detachment, and **(E)** IFN-γ for acute retinal necrosis. *$P < 0.05$, **$P < 0.001$ (Mann–Whitney U test).

obtained directly via ocular puncture, allowing direct analysis of the vitreous.

Although this study has revealed several interesting findings, there are also several limitations. First, the evaluation of machine learning algorithms for predicting diagnosis of intraocular diseases was constrained by the single-center design, retrospective data, variations in sampling time within the cohort, and a relatively small sample size. Because the efficacy of machine learning techniques often improves with larger datasets, future investigations involving more extensive patient cohorts with more diverse intraocular diseases are necessary to validate the diagnostic significance of the immune mediators identified in this study. Therefore multicenter research to provide additional data and include other intraocular diseases could enhance the predictive accuracy and classification reliability.

Second, the study lacked specific subject inclusion and exclusion criteria such as disease type and severity, ongoing therapy, and duration between disease onset and vitreous sampling. This could potentially impact the immune mediator profile, possibly with undesirable effect. For example, the majority of the patients with vitreoretinal lymphoma, sarcoidosis, and uveitis of unknown cause had received steroid treatments including eyedrops, sub-Tenon's injections, and oral medications for intraocular inflammation during the period leading up to one month before vitrectomy. These interventions might have influenced the vitreous samples. While an ideal study would recruit eyes that have not undergone any prior treatment, it is common in clinical practice to analyze vitreous samples from patients who had recently received corticosteroid treatment. Despite these limitations, we believe that this study provides valuable real-world data.

Third, although rhegmatogenous retinal detachment, proliferative diabetic retinopathy, retinal vein occlusion, idiopathic macular hole, idiopathic epiretinal membrane, retinal hemangioma, and melanoma are not intraocular diseases usually diagnosed using vitreous humor collected at diagnostic vitrectomy, analyzing a panel of immune factors in the vitreous by machine learning is valuable for the classification of various intraocular diseases and may identify biomarkers corresponding to specific intraocular diseases.

Fourth, unlike our previous study of aqueous humor cytokine profiles,[5] the present study of vitreous samples lacks a control group. Although eyes undergoing cataract surgery are commonly used as controls, it is unethical to use normal vitreous in research. In this study, eyes with idiopathic epiretinal membrane could have served as potential controls.

Finally, external validation was not conducted. The whole dataset was split into training and test data for creating the RF classifier models, and no new dataset was available as external validation data. Nevertheless, we demonstrated the applicability of machine training-based classification for predicting diagnosis of intraocular diseases in a large and diverse cohort of patients. Our results shed light on the considerable heterogeneity of immune mediator responses associated with individual diseases, and it is noteworthy that the findings could be informative both in determining causative factors and understanding the consequences. In summary, the findings contribute to a deeper understanding of the mechanisms underlying the pathogenesis of intraocular diseases and may potentially inform the methodological design of future machine learning classifiers.

In conclusion, the present research demonstrates the successful prediction of specific intraocular diseases including vitreoretinal lymphoma, endophthalmitis, and uveal melanoma using RF algorithms applied to 28 vitreous immune mediators. The results may contribute to enhance our understanding of the pathophysiology of various intraocular diseases, and propose emerging diagnostic and therapeutic targets. Furthermore, the findings from this

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study may encourage further research aiming at uncovering novel molecular mechanisms and diagnostic capabilities of immune mediators. This, in turn, may contribute to the identification of innovative therapeutic targets for specific intraocular diseases.

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