

A Blood Protein Signature Stratifies Clinical Response to csDMARD Therapy in Pediatric Uveitis

Roos A. W. Wennink^{1,2}, Viera Kalinina Ayuso¹, Weiyang Tao², Eveline M. Delemarre², Joke H. de Boer¹, and Jonas J. W. Kuiper^{1,2}

¹ Department of Ophthalmology, University Medical Center Utrecht, Utrecht University, The Netherlands

² Center for Translational Immunology, University Medical Center Utrecht, Utrecht University, The Netherlands

Correspondence: Roos A.W. Wennink, University Medical Center Utrecht, Department of Ophthalmology, E03.136, PO Box 85500, 3508 GA Utrecht, The Netherlands.
e-mail: ra.w.wennink@umcutrecht.nl

Received: October 13, 2021

Accepted: January 4, 2022

Published: February 2, 2022

Keywords: pediatric uveitis; biomarker; treatment response

Citation: Wennink RAW, Kalinina Ayuso V, Tao W, Delemarre EM, de Boer JH, Kuiper JJW. A blood protein signature stratifies clinical response to csDMARD therapy in pediatric uveitis. *Transl Vis Sci Technol.* 2022;11(2):4.
<https://doi.org/10.1167/tvst.11.2.4>

Purpose: To identify a serum biomarker signature that can help predict response to conventional synthetic disease-modifying antirheumatic drug (csDMARD) therapy in pediatric noninfectious uveitis.

Methods: In this case-control cohort study, we performed a 368-plex proteomic analysis of serum samples of 72 treatment-free patients with active uveitis (new onset or relapse) and 15 healthy controls. Among these, 37 patients were sampled at diagnosis before commencing csDMARD therapy. After 6 months, csDMARD response was evaluated and cases were categorized as “responder” or “nonresponder.” Patients were considered “nonresponders” if remission was not achieved under csDMARD therapy. Serum protein profiles were used to train random forest models to predict csDMARD failure and compared to a model based on eight clinical parameters at diagnosis (e.g., maximum cell grade).

Results: In total, 19 of 37 (51%) cases were categorized as csDMARD nonresponders. We identified a 10-protein signature that could predict csDMARD failure with an overall accuracy of 84%, which was higher compared to a model based on eight clinical parameters (73% accuracy). Adjusting for age, sex, anatomic location of uveitis, and cell grade, cases stratified by the 10-protein signature at diagnosis showed a large difference in risk for csDMARD failure (hazard ratio, 12.8; 95% confidence interval, 2.5–64.6; $P = 0.002$).

Conclusions: Machine learning models based on the serum proteome can stratify pediatric patients with uveitis at high risk for csDMARD failure.

Translational Relevance: The identified protein signature has implications for the development of clinical decision tools that integrate clinical parameters with biological data to better predict the best treatment option.

Introduction

Noninfectious pediatric uveitis accounts for 5% to 10% of all patients with uveitis.¹ Despite this relatively small proportion, it has often a severe disease course and a disproportionately large disease burden due to the young age of onset.^{2,3} Direct evidence is lacking, but an immune-mediated etiology is strongly suspected in noninfectious pediatric uveitis.^{4–9} Over half of cases have chronic disease and consequently require long periods of treatment with immunosuppressive agents, most often conventional synthetic disease-modifying antirheumatic drugs (csDMARDs)

such as methotrexate or mycophenolate mofetil.^{10–12} Of note is that early start of csDMARD therapy is generally associated with a more beneficial disease outcome.^{13–15} Nevertheless, in approximately a third of cases, csDMARDs fail to control uveitis, and adding therapy with biologics—usually tumor necrosis factor α inhibitors—is required.^{10,16–25} It is currently not possible to objectively predict the response to csDMARD in advance; thus, with the current treatment guidelines, it may take a considerable amount of time for a group of patients to receive their optimal therapeutic regimen, all while being exposed to the visual-threatening risks of undertreatment or potential side effects of overtreatment. The early identification

of patients in whom csDMARDs will not sufficiently control eye inflammation will help mitigate these risks. Identification of these cases may be achieved by discovery of predictive immune-related protein signatures in cases that require biologics (csDMARD failure) versus cases that do not. Studies in patients with other inflammatory diseases have shown that blood protein profiling has the potential to predict treatment response before commencing treatment.²⁶ In this study, we performed immune profiling of a cohort of children with noninfectious uveitis with ophthalmologic follow-up to identify key proteomic profiles that can stratify response to csDMARDs in advance on a personalized basis.

Methods

Patient and Material Collection

The study was approved by the Medical Ethical Research Committee of the University Medical Center Utrecht in concordance with the Declaration of Helsinki principles. Written informed consent was obtained from all patients aged ≥ 18 years, from both parents and patients in cases between 12 and 18 years of age, as well as from parents in cases < 12 years old. Peripheral blood samples (BD Vacutainer serum tubes; BD, USA) were obtained from patients with juvenile idiopathic arthritis-associated uveitis (JIA-U, $n = 14$), idiopathic chronic anterior uveitis (i.e., no JIA) ($n = 7$), (*HLA-B27* positive) acute anterior uveitis ($n = 7$), intermediate uveitis ($n = 11$), and panuveitis ($n = 33$). Serum samples of 15 healthy controls (i.e., without a history of inflammatory eye disease) were collected from children without uveitis during surgery indicated for strabismus. Samples were collected at the outpatient department (uveitis biobank, $n = 48$) or were the remainder of samples obtained for diagnostic purposes (diagnostic laboratory, $n = 39$). All patients had active uveitis (new onset or relapse), and none of the patients received immunomodulatory treatment at the time of sampling. The diagnosis of uveitis was established by a trained uveitis specialist according to the standardization of uveitis (SUN) criteria.²⁷ All cases were recruited at the University Medical Center Utrecht, the Netherlands (tertiary referral center and National Uveitis Center of Excellence). All samples were obtained from patients with a diagnosis of noninfectious uveitis before the age of 18 years. JIA was diagnosed according to the criteria of the International League of Associations for Rheumatology or by former criteria (e.g., European League Against Rheumatism).^{28,29} Patients with JIA were screened by an ophthalmologist according to the

guidelines of the Academy of Pediatrics.^{30,31} Patients with one or more cells in the anterior chamber and treated with at least topical steroids were diagnosed with JIA-U. Patients using csDMARDs ($n = 37$) were categorized as responders and nonresponders. Patients were considered “responders” if inactive disease was achieved by csDMARD therapy (i.e., $\leq 1+$ cells in aqueous humor and/or vitreous body after 6 months and with systemic corticosteroids ≤ 7.5 mg). Patients were considered “nonresponders” if remission was not achieved under csDMARD therapy and addition of a biological was indicated. This was defined as the presence of any of the following features:

- 1) $\geq 3+$ cells in aqueous humor and/or vitreous body after 4 months
- 2) $\geq 2+$ cells after 6 months and/or disease activity on fluorescein angiography
- 3) Necessity of use of systemic corticosteroids ≥ 7.5 mg/d³²

Serum Proteomic Olink Analysis

After blood withdrawal, serum tubes were kept for 30 minutes at room temperature and immediately centrifuged at $2000 \times g$ for 10 minutes at room temperature. Serum was collected and stored directly at -80°C . Frozen serum samples were shipped on dry ice to Olink Proteomics (Uppsala, Sweden) without prior thawing and measured using the proximity extension assay (PEA) technology based on Proseek Multiplex panel (Olink Proteomics).^{33,34} In short, PEA technology uses a set of antibodies that are linked with matching DNA-oligonucleotides per protein. After binding the protein, the oligonucleotides hybridize when brought into proximity and are extended by DNA polymerase, forming polymerase chain reaction (PCR) targets. Using next-generation sequencing, the PCR targets are quantified. The Olink Explore 384 inflammation panel was used to measure 368 proteins associated with inflammatory responses. The serum protein expression data from the Olink platform are expressed as an arbitrary unit (Normalized Protein eXpression [NPX]) representing the relative protein concentration based on a \log_2 scale. The full list of protein targets ($n = 368$) is provided in Supplementary Table S1.

Data Analysis and Statistics

Statistical analyses were performed in R (version 3.6.1). The statistical analysis of proteomic data was performed on protein expression data (NPX units).

We used the k -nearest neighbors algorithm to impute missing values (6.9%; interquartile range, 5.7–8.0) with $k = 5$ with the R package *Hmsic*. Principal component analysis was performed using the *factoextra* package in R, and batch effects (uveitis biobank versus diagnostic laboratory) were removed by the *ComBat* (empirical Bayes) method implemented in the *sva* package in R (Supplementary Fig. S1).^{35,36} Differential expression analyses on batch-corrected data were conducted using a likelihood ratio test, and the proteins with a nominal $P < 0.05$ were considered differentially expressed proteins. Fisher's exact test or Pearson's χ^2 test was used to compare categorical variables between patients with uveitis and controls, or between responders and nonresponders. The Wilcoxon rank-sum test was used for continuous variables.

Prediction of csDMARD Response by Machine Learning Models

We built supervised machine learning models to predict response to csDMARDs (responders versus nonresponders). Four machine learning models were built based on the random forest algorithm using the *randomForest* R package.³⁷ The first model was designed using ophthalmologic clinical data at diagnosis (model 1). (1) Age of uveitis onset, (2) sex, (3) laterality of uveitis, (4) maximum cell grade in the aqueous humor and/or vitreous body (depending on site of inflammation), (5) a complication at diagnosis (i.e., band keratopathy, posterior synechiae, or cataract), (6) antinuclear antibody (ANA) seropositivity, (7) measurement of the central macular thickness (CMT) in micrometers on optical coherence tomography (OCT) imaging, and (8) measurement of retinal nerve fiber layer thickness (RNFL) in micrometers on OCT imaging were included as features (i.e., predictors), and the response to csDMARD was considered as output of the random forest model. The cell grade in the aqueous humor was scored according to the SUN criteria by an ophthalmologist specialized in pediatric uveitis.²⁷ The identical scale was applied for grading cells in the vitreous body through a dilated eye (i.e., vitreous cell grade based on SUN criteria for aqueous humor scoring with a 1-mm \times 1-mm slit beam). In case of bilateral uveitis, the eye with the worst score was used for the model. Missing values in ANA seropositivity ($n = 1$), measurement of CMT ($n = 6$), and measurement of RNFL ($n = 13$) were imputed using the *randomForest* package in R.³⁷ A second model was based on the full serum protein profile ($n = 368$ proteins) of responders and nonresponders (model 2). The 10 most important features (feature importance

metric) were selected and used to run a third random forest model (model 3). The last model was a combination of model 1, the clinical parameters, and model 3, the 10-protein signature (model 4). The number of variables used at each split (mtry) with the lowest out-of-bag error was selected for each model (mtry = 3, 19, 4, and 4, for models 1, 2, 3, and 4, respectively). The number of trees was set at 5000 for all models. The performance of the models was evaluated using leave-one-out cross-validation (i.e., the number of folds equals the number of samples, and for every fold, one sample is used as the test set and the rest as the training set). The overall accuracy, true-positive rate, and true-negative rate are evaluated based on the test set.

To establish the relative difference in risk profiles captured by the 10-protein signature, we stratified the 37 patients with csDMARD into two clusters based on the first principal component of the expression profile of the 10 proteins. To determine the optimal cutoff value, we used the *surv_cutpoint* function of the *survminer* R package with a minimal proportion of observations of 0.4.³⁸ The cumulative hazard was plotted using the *survival* R package and analyzed using *coxph* and *ggforest* from the *survival* and *survminer* R packages, respectively.^{38,39}

Results

In total, 72 cases with pediatric uveitis were included in this study. Demographic characteristics of the study patients are shown in Table 1. No significant differences in sex and age were observed between patients with uveitis and healthy controls. Targeted proteomics of serum was conducted in all cases and control samples. In total, 368 unique proteins were used for analysis.

Comparison of the serum proteome of uveitis cases versus controls identified 62 differentially expressed proteins ($P < 0.05$), of which ARHGEF12 (rho guanine nucleotide exchange factor 12), PRKAB1 (protein kinase AMP-activated noncatalytic subunit beta 1), and DECR1 (2,4-dienoyl-CoA reductase) were the most differentially expressed (Supplementary Table S2 and Fig. 1A). However, the levels of these proteins varied substantially between cases regardless of uveitis subtype (Fig. 1B), suggestive of molecular heterogeneity linked to features beyond uveitis entity (i.e., treatment response).

To explore this in more detail, we assessed the proteomic differences between csDMARD responders and nonresponders. First, we filtered for patients who were sampled at diagnosis (46/72) and started csDMARD treatment after sampling (37/46). Of the

Table 1. Demographics and Baseline Characteristics of the Study Patients (*n* = 87)

Characteristic	Uveitis Cases	Controls	P Value
N	72	15	
Male, <i>n</i> (%)	26 (36)	6 (40)	0.78
Age at sampling, median (IQR), y	13 (10–15)	12 (5–27)	0.87
ANA seropositivity, <i>n</i> (%)	35 (50)	NA	NA
Age at uveitis diagnosis, median (IQR), y	11 (8–14)	NA	NA
Duration of uveitis, median (IQR), y	0.13 (0.01–0.70)	NA	NA

ANA, antinuclear antibody; IQR, interquartile range; NA, not applicable.

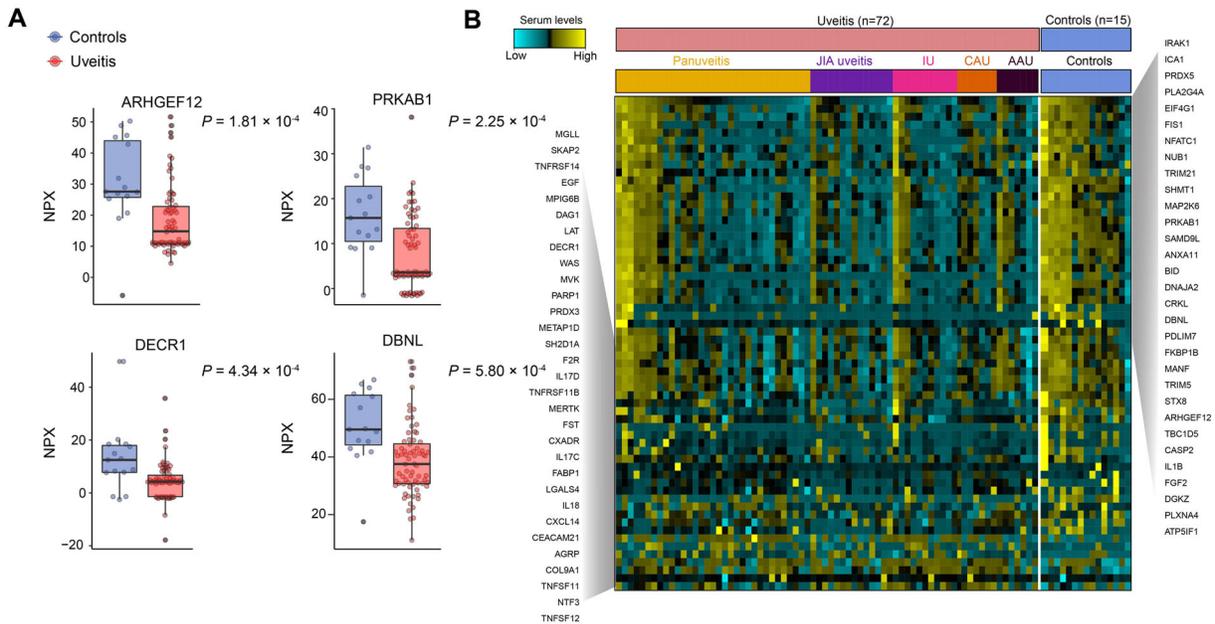


Figure 1. Serum protein analysis of pediatric uveitis (*n* = 72) and healthy controls (*n* = 15). **(A)** Scatterplots and boxplots of the top four most significantly different serum proteins between uveitis cases and healthy controls. Protein expression data and details on statistical analysis for each protein analyte (*n* = 368) are shown in Supplementary Table S2. **(B)** Heatmap of the 62 differently expressed proteins between uveitis cases and controls. The levels for each protein analyte are shown for each of the samples in the study and are color-coded from low (cyan) to high (yellow). ARHGEF12, rho guanine nucleotide exchange factor 12; DBNL, drebrin-like protein; DECR1, 2,4-dienoyl-CoA reductase 1; PRKAB1, protein kinase AMP-activated noncatalytic subunit beta 1.

37 patients who started csDMARD during follow-up, 19 of 37 (51%) cases required anti-tumor necrosis factor α (TNF α) therapy in addition to csDMARD for disease control, which we considered “nonresponders” to csDMARD therapy (see Methods and Fig. 2A). There was a moderate difference in the time to start with csDMARD nonresponders and the other 18 “csDMARD responders” (1 month versus 2.6 months, $P = 0.049$, Table 2). In addition, as expected, the cell grade in aqueous humor and/or vitreous body was higher in nonresponders (1+ cells versus 3+ cells, $P = 0.003$). No other differences were observed between the two groups (Table 2). The proteomic profile of csDMARD responders versus nonresponders was

compared. The protein expression data of responders and nonresponders are shown in Supplementary Table S3. We built four machine learning models based on the random forest algorithm to predict csDMARD response using the clinical parameters at diagnosis, serum proteome (*n* = 368), a top 10-protein signature (i.e., the 10 most important features in the serum proteome random forest model), and a model using the clinical parameters and the top 10-protein signature. A 10-protein model showed an overall accuracy of 84% (95% confidence interval [CI], 68–94%), which was higher compared to a model based on the clinical parameters (Figs. 2B, 2C). The true-positive and true-negative rates of this model were 78% and 89%,

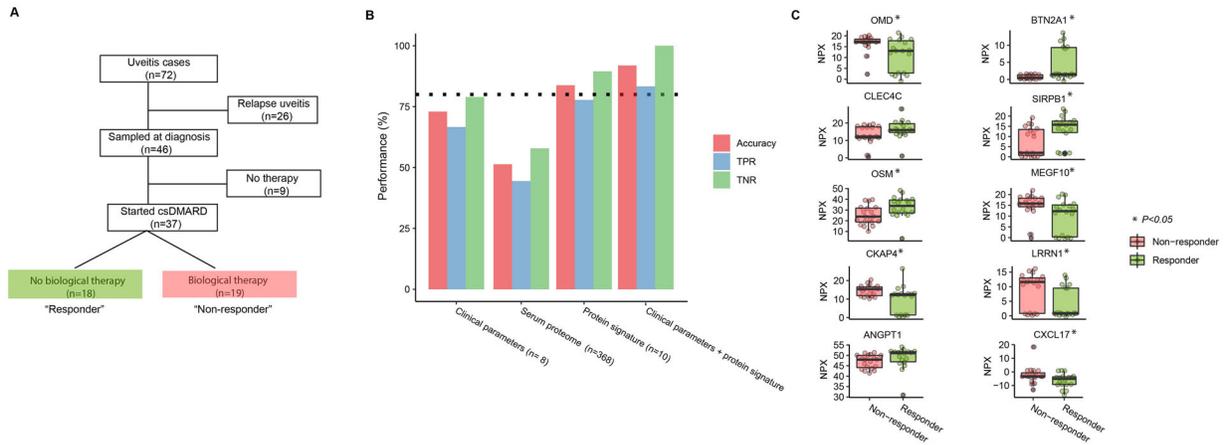


Figure 2. Random forest models performance for classification of csDMARD response in pediatric uveitis. **(A)** A flowchart indicating the selection of patients for analysis of csDMARD response at diagnosis (new-onset uveitis). **(B)** The accuracy, true-negative rate, and true-positive rate of the random forest models based on eight clinical parameters (model 1), the serum proteome (model 2, $n = 368$ proteins), the 10-protein signature (model 3), and the combination of clinical parameters and the 10-protein signature (model 4). The horizontal dotted line indicates a threshold accuracy of 80%. **(C)** Boxplots of top 10 most important proteins that distinguish responders from nonresponders identified by random forest model 2. The proteins are sorted based on importance from left to right (feature importance metric). The asterisk indicates a $P < 0.05$ from the likelihood ratio test between csDMARD responders and nonresponders. TNR, true-negative rate; TPR, true-positive rate.

respectively. Among the proteins, the top three proteins that drove this signature were osteomodulin, oncostatin M (OSM), and the plasmacytoid dendritic cell-associated protein C-type lectin domain family 4 member C (CLEC4C). Adding the top 10-protein signature to the model with clinical parameters improved the overall accuracy from 73% to 92%, indicating improvement of adding proteins into a classification model based on clinical features only (Fig. 2B).

To establish the relative difference in risk profiles captured by this 10-protein signature, we split the 37 patients with csDMARD into two clusters based on the levels of the 10 proteins (using the first principal component of the expression data) (Fig. 3A and Supplementary Table S4). Cluster 1 consisted primarily of nonresponders (15/17) and cluster 2 mainly of responders (16/20). Survival analysis showed that cases stratified upon the 10-protein signature differed significantly in their probability for csDMARD failure (log rank test = 2.28×10^{-5}). At 9 months after diagnosis, half of the cases in cluster 1 failed on a csDMARD compared to 15% cases in cluster 2 (Fig. 3B). Multivariate Cox proportional hazard analysis adjusting for age, sex, anatomic location of uveitis, and cell grade in aqueous humor and/or vitreous body (as a proxy for uveitis activity) revealed that patients in cluster 1 ($n = 17$) had a substantially increased risk for being a “nonresponder” compared to cases in cluster 2 ($n = 20$) (hazard ratio, 12.8; 95% CI, 2.5–64.6; $P = 0.002$)

(Fig. 3C). These data indicate that a proteomic profile can be harvested to predict risk categories for csDMARD failure in patients with pediatric uveitis.

Discussion

Here we used targeted proteomic profiling of serum samples drawn from children with active noninfectious uveitis in order to identify a protein signature by diagnosis that stratifies cases that have a high risk for csDMARD failure during follow-up. These results show that a 10-protein signature was highly predictive for csDMARD failure in children with noninfectious uveitis. The identified protein signature will guide us to the realization of personalized medicine for children with noninfectious uveitis.

Treatment guidelines for pediatric uveitis, except for JIA-U, are sparse and heavily dependent on individual clinical monitoring of disease.^{18,22–25} This is in part due to the limited number of clinical trials in this patient population and the lack of biomarker studies for the prediction of treatment response.^{19–21} Ideally, clinical decision tools (i.e., algorithms) integrate clinical parameters with biological data to better predict the best treatment option, which considers prompt disease control, the least adverse drug effects, and the possibility to reduce the disease burden. Here, we provide a proof of concept for stratifying the response

Table 2. Baseline and Clinical Characteristics at Diagnosis of Responders ($n = 18$) and Nonresponders ($n = 19$)

Characteristic	Responder	Nonresponder	<i>P</i> Value
<i>N</i> (%)	18 (49)	19 (51)	
Male, <i>n</i> (%)	4 (22)	8 (42)	0.20
Age at uveitis onset, median (IQR), y	13 (11–15)	10 (8–13)	0.08
Bilateral, <i>n</i> (%)	16 (89)	12 (63)	0.12
Location of uveitis, <i>n</i> (%)			0.80
Anterior uveitis	5 (28)	6 (32)	
Nonanterior uveitis	13 (72)	13 (68)	
Maximum cell grade aqueous humor and/or vitreous body, ^a median (IQR)	1 (1–3)	3 (3–4)	0.003
Measurement of central macular thickness, median (IQR), μm	296 (265–338)	348 (266–399)	0.17
Measurement of retinal nerve fiber layer thickness, median (IQR), μm	193 (123–204)	197 (168–219)	0.32
Complications, ^b <i>n</i> (%)	9 (50)	9 (47)	0.87
ANA seropositivity, <i>n</i> (%)	6 (35)	10 (53)	0.24
Type of csDMARD, <i>n</i> (%)			0.40
Methotrexate	10 (53)	11 (61)	
Mycophenolate mofetil	9 (47)	7 (39)	
Time to start csDMARD, median (IQR), mo	2.6 (0.7–3.4)	1.0 (0.4–2.0)	4.99×10^{-2}

^aIn patients with anterior uveitis ($n = 11$), the cell grade in the aqueous humor was scored according to the SUN criteria and was used for the analysis.²⁷ For patients with nonanterior uveitis ($n = 26$), the identical scale (i.e., SUN criteria) was applied for grading cells in the aqueous humor and in the vitreous body. The site with the highest cell grade was used for the analysis. In 10 of 26 patients with nonanterior uveitis, the maximum cell grade in the vitreous body was used for the analysis.

^bComplications at diagnosis: band keratopathy, posterior synechiae, or cataract.

to csDMARD in children with noninfectious uveitis using a blood protein signature.

There are, however, a number of considerations and limitations of the current study, which we outline in detail. In our cohort, half of cases had an indication for anti-TNF α therapy in addition to a csDMARD, which is higher than reported in literature ($\sim 30\%$).^{10,16,17} However, patients referred to a tertiary center hospital, as in our study, are 1.6 times more likely to be treated with biological therapy when compared to patients referred to primary care, which may explain the higher rate.⁴⁰ In addition, biological therapy can also be initiated to prevent long-term use of systemic corticosteroids. The high number of patients with refractory uveitis and the beneficial therapeutic effect of biologics in these cases emphasize the unmet need for early identification of csDMARD failure.^{19–21,41} A parsimonious model based on the 10 most discriminative proteins showed the highest overall accuracy. Patients stratified by the expression levels of these 10 proteins showed a large difference in risk for csDMARD failure. Although the proportion of each uveitis subtype in our cohort did not reflect the population at larger (i.e., anterior uveitis is by far the most common type seen in

clinics), our analysis revealed that the protein signature stratified patients independent of anatomic location of uveitis. We therefore consider that these results are applicable to the wider population of noninfectious uveitis cases. Although we observed the influence of panuveitis and treatment response to csDMARD as an interacting covariate in a multivariate Cox model (including also age, sex, and cell grade) (Fig. 3C), Cox proportional analysis assessing the overall relationship between anatomic location of uveitis and csDMARD response did not support an association with panuveitis ($P = 0.76$). Also, the median time to csDMARD was shorter in nonresponders compared to responders (1 month versus 2.6 months). When adding the time to csDMARD as a covariate to the Cox model (Cox proportional hazard analysis adjusting for age, sex, anatomic location, cell grade, and time to csDMARD), we found the predictive capacity of the serum 10-protein signature to be modestly affected (Cox $P_{10\text{-protein} + \text{age} + \text{sex} + \text{location} + \text{cell grade}} = 0.002$, Cox $P_{10\text{-protein} + \text{age} + \text{sex} + \text{location} + \text{cell grade} + \text{time to csDMARD}} = 0.08$), which supports that the 10-protein signature has clinical potential to predict csDMARD response. Four of 19 nonresponders (21%) were clustered with

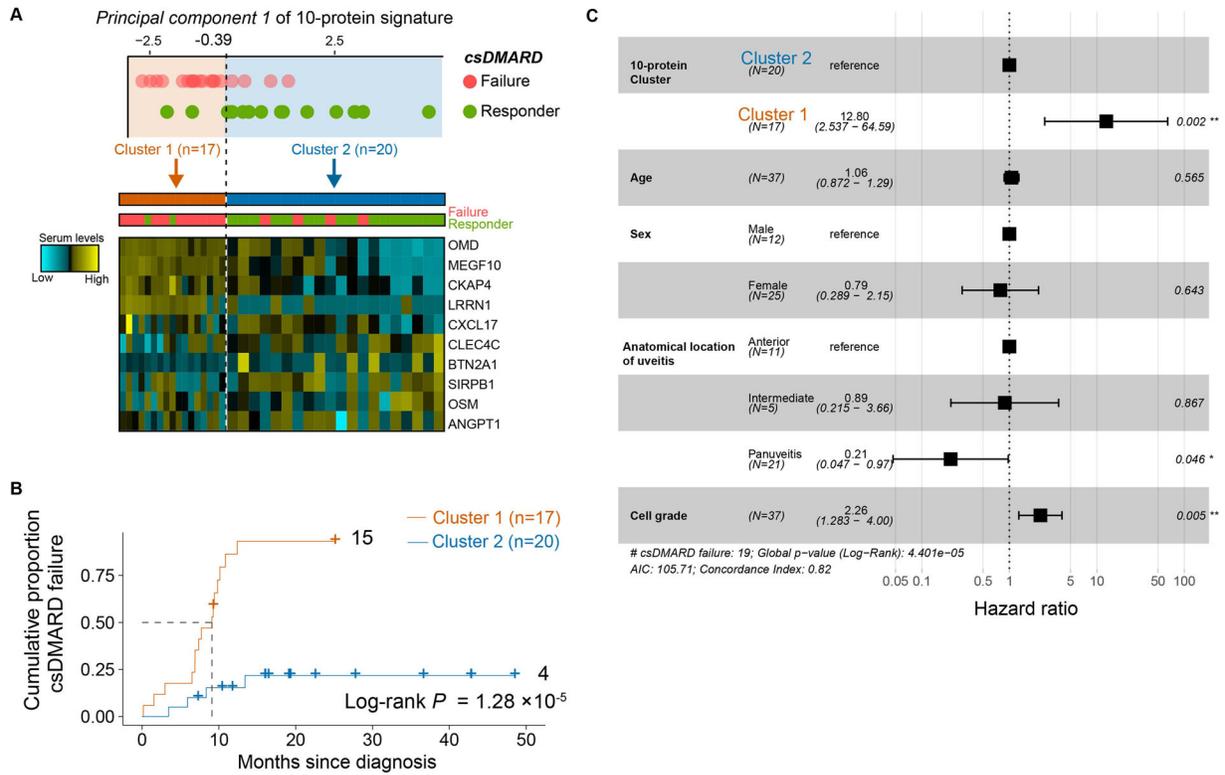


Figure 3. A 10-protein signature stratifies cases with high risk for csDMARD failure at pediatric uveitis diagnosis. **(A)** Two clusters are identified based on the first principal component of the expression of the 10 serum proteins identified by random forest model 2 (dotted line). The relative levels of the 10 serum proteins for each case in both clusters are color-coded from low (cyan) to high (yellow). **(B)** The cumulative event curve for csDMARD failure for each of the two 10-protein clusters identified in panel A. The dotted line indicates the time to 50% csDMARD failure in cluster 1. **(C)** A forest plot of the multivariate Cox model used to assess the proportional hazard for csDMARD failure for each cluster identified in panel A. Hazard was adjusted for age, sex, anatomic location of uveitis, and cell grade in the aqueous humor and/or vitreous body. **p* value < 0.05 and ***p* value < 0.01.

responders, illustrating that, although a vast improvement over current state of the art (i.e., unable to predict csDMARD response), our algorithm is not perfect and requires prospective validation to determine the precise accuracy and robustness in a “real-life” setting. Regardless, it is of interest to note that this signature contained proteins linked to uveitis biology and treatment response in other inflammatory diseases. For example, OSM is reported as a biomarker for treatment response in patients with inflammatory bowel disease, and CLEC4C is a hallmark protein of plasmacytoid dendritic cells that are implicated in noninfectious uveitis.^{42,43}

Although we determined that the 10-protein signature was found to be higher than the clinical model, we would like to emphasize that the model based on eight clinical parameters was also relatively accurate for prediction of csDMARD failure. These eight clinical parameters are also part of the now widely used SUN criteria for noninfectious uveitis assessment. However, we deliberately left out posterior segment imaging,

such as fluorescein angiography scores, because these imaging data would not be available for most patients with anterior uveitis. The lack of an objective disease severity marker available across all types of noninfectious uveitis makes predictive modeling challenging. To overcome this, we considered taking a parameter with a relatively similar context of information that is available for most cases (vitreous haze was not scored in all patients). In our opinion, “number of cells” at diagnosis may serve as a solution to this problem where we used the highest cell score in either aqueous or vitreous fluid to be able to conduct analyses for all cases of noninfectious uveitis. Consequently, for anterior uveitis, this means that the disease severity was scored according to the SUN criteria (i.e., number of cells in the anterior chamber), while vitreous cell score for nonanterior uveitis deviated from these criteria. Regardless, we demonstrate that this parameter (i.e., the maximum cell grade in the anterior chamber and/or vitreous body at diagnosis) was the most discriminative clinical feature for csDMARD

response according to random forest analysis. Also, we noted a relationship between csDMARD response and cell grade using multivariate Cox proportional hazard analysis (Fig. 3C). It is tempting to speculate that a higher intraocular cell grade reflects a high disease activity and severity, which requires more often biological therapy to control inflammation. These results underscore that the standardized SUN criteria can stratify patients at high risk for csDMARD failure. In this study, however, we extend this and show proof of concept that blood biomarkers can further (i.e., independently) improve the prediction of csDMARD failure at diagnosis. A model based on the clinical parameters and the 10-protein signature improved the detection of csDMARD failure from 73% to 92%, and this supports that adding molecular biomarkers in blood can significantly improve early detection of treatment failure in pediatric uveitis. Future studies should aim to integrate a wider variety of clinical parameters with precise cutoff levels for the protein biomarkers to generate a clinical decision algorithm for the stratification of patients with a high risk for csDMARD failure.

In conclusion, we showed that a proteomic signature detectable at diagnosis could accurately stratify csDMARD response. Future studies should be focusing on the clinical implication of the use of proteomics in combination with the clinical observations in predicting treatment response and understanding the mechanism of how different patients respond to immunosuppressive therapy, paving the path toward personalized medicine.

Acknowledgments

Supported by the Dutch Ophthalmology Foundation “UitZicht” and ODAS stichting.

Disclosure: **R.A.W. Wennink**, Bayer (F); **V. Kalinina Ayuso**, Bayer (F); **W. Tao**, Bayer (F); **E.M. Delemarre**, Bayer (F); **J.H. de Boer**, Bayer (F); **J.J.W. Kuiper**, Bayer (F)

References

1. Wakefield D, Chang JH. Epidemiology of uveitis. *Int Ophthalmol Clin*. 2005;45(2):1–13.
2. Wieringa WG, van Berkel RJ, Los LI, Lelieveld OTHM, Armbrust W. Physical and psychosocial health in pediatric uveitis patients [published online July 6, 2021]. *Ocul Immunol Inflamm*.
3. McDonald J, Cassedy A, Altaye M, et al. Comprehensive assessment of quality of life, functioning and mental health in children with juvenile idiopathic arthritis and non-infectious uveitis [published online January 9, 2021]. *Arthritis Care Res (Hoboken)*.
4. Walscheid K, Neekamp L, Heiligenhaus A, Weinhage T, Heinz C, Foell D. Increased circulating proinflammatory T lymphocytes in children with different forms of anterior uveitis: results from a pilot study. *Ocul Immunol Inflamm*. 2019;27(5):788–797.
5. Walscheid K, Neekamp L, Heiligenhaus A, et al. Peripheral blood monocytes reveal an activated phenotype in pediatric uveitis. *Clin Immunol*. 2018;190:84–88.
6. Kalinina Ayuso V, Makhotkina N, van Tent-Hoeve M, et al. Pathogenesis of juvenile idiopathic arthritis associated uveitis: the known and unknown. *Surv Ophthalmol*. 2014;59(5):517–531.
7. Wennink RAW, Pandit A, Haasnoot AJW, et al. Whole transcriptome analysis reveals heterogeneity in B cell memory populations in patients with juvenile idiopathic arthritis-associated uveitis. *Front Immunol*. 2020;11:2170.
8. Kalinina Ayuso V, van Dijk MR, de Boer JH. Infiltration of plasma cells in the iris of children with ANA-positive anterior uveitis. *Invest Ophthalmol Vis Sci*. 2015;56(11):6770–6778.
9. Wildschütz L, Ackermann D, Witten A, et al. Transcriptomic and proteomic analysis of iris tissue and aqueous humor in juvenile idiopathic arthritis-associated uveitis. *J Autoimmun*. 2019;100:75–83.
10. Henderson LA, Zurakowski D, ST Angeles-Han, et al. Medication use in juvenile uveitis patients enrolled in the Childhood Arthritis and Rheumatology Research Alliance Registry. *Pediatr Rheumatol Online J*. 2016;14(1):9.
11. Foeldvari I, Wierk A. Methotrexate is an effective treatment for chronic uveitis associated with juvenile idiopathic arthritis. *J Rheumatol*. 2005;32(2):362–365.
12. Doycheva D, Deuter C, Stuebiger N, Biester S, Zierhut M. Mycophenolate mofetil in the treatment of uveitis in children. *Br J Ophthalmol*. 2007;91(2):180–184.
13. 2nd Gregory AC, JH Kempen, Daniel E, et al. Risk factors for loss of visual acuity among patients with uveitis associated with juvenile idiopathic arthritis: the Systemic Immunosuppressive Therapy for Eye Diseases Study. *Ophthalmology*. 2013;120(1):186–192.
14. Cheung CSY, Mireskandari K, Ali A, Silverman E, Tehrani N. Earlier use of systemic

- immunosuppression is associated with fewer ophthalmic surgeries in paediatric non-infectious uveitis. *Br J Ophthalmol*. 2020;104(7):938–942.
15. Sijssens KM, Rothova A, Van De Vijver DA, Stilma JS, De Boer JH. Risk factors for the development of cataract requiring surgery in uveitis associated with juvenile idiopathic arthritis. *Am J Ophthalmol*. 2007;144(4):574–579.
 16. McCracken C, Yeh S, Jenkins K, et al. Timing of infliximab and adalimumab initiation despite methotrexate in children with chronic non-infectious anterior uveitis. *Eye (Lond)*. 2019;33(4):629–639.
 17. Simonini G, Paudyal P, Jones GT, Cimaz R, Macfarlane GJ. Current evidence of methotrexate efficacy in childhood chronic uveitis: a systematic review and meta-analysis approach. *Rheumatology (Oxford)*. 2013;52(5):825–831.
 18. Angeles-Han ST, Lo MS, Henderson LA, et al.; Juvenile Idiopathic Arthritis Disease-Specific and Uveitis Subcommittee of the Childhood Arthritis Rheumatology and Research Alliance. Childhood arthritis and rheumatology research alliance consensus treatment plans for juvenile idiopathic arthritis-associated and idiopathic chronic anterior uveitis. *Arthritis Care Res (Hoboken)*. 2019;71:482–491.
 19. Ramanan AV, Dick AD, Jones AP, et al. Adalimumab plus methotrexate for uveitis in juvenile idiopathic arthritis. *N Engl J Med*. 2017;376:1637–1646.
 20. Ramanan AV, Dick AD, Jones AP, et al. Adalimumab in combination with methotrexate for refractory uveitis associated with juvenile idiopathic arthritis: a RCT. *Health Technol Assess*. 2019;23:1–140.
 21. Quartier P, Baptiste A, Despert V, et al. ADJUVITE Study Group. ADJUVITE: a double-blind, randomised, placebo-controlled trial of adalimumab in early onset, chronic, juvenile idiopathic arthritis-associated anterior uveitis. *Ann Rheum Dis*. 2018;77:1003–1011.
 22. Heiligenhaus A, Minden K, Tappeiner C, et al. Update of the evidence based, interdisciplinary guideline for anti-inflammatory treatment of uveitis associated with juvenile idiopathic arthritis. *Semin Arthritis Rheum*. 2019;49:43–55.
 23. Angeles-Han ST, Ringold S, Beukelman T, et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the screening, monitoring, and treatment of juvenile idiopathic arthritis-associated uveitis. *Arthritis Care Res (Hoboken)*. 2019;71:703–716.
 24. Dick AD, Rosenbaum JT, Al-Dhibi HA, et al. Fundamentals of Care for Uveitis International Consensus Group. Guidance on noncorticosteroid systemic immunomodulatory therapy in noninfectious uveitis: Fundamentals of Care for Uveitis (FOCUS) Initiative. *Ophthalmology*. 2018;125:757–773.
 25. Constantin T, Foeldvari I, Anton J, et al. Consensus-based recommendations for the management of uveitis associated with juvenile idiopathic arthritis: the SHARE initiative. *Ann Rheum Dis*. 2018;77(8):1107–1117.
 26. van der Heijden EHM, Blokland SLM, Hillen MRH, et al. Leflunomide–hydroxychloroquine combination therapy in patients with primary Sjögren’s syndrome (RepurpSS-I): a placebo-controlled, double-blinded, randomised clinical trial. *Lancet Rheumatol*. 2020;2(5):e260–e269.
 27. Jabs DA, Nussenblatt RB, Rosenbaum JT; Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data: results of the first international workshop. *Am J Ophthalmol*. 2005;140(3):509–516.
 28. Petty RE, Southwood TR, Manners P, et al. International League of Associations for Rheumatology classification of juvenile idiopathic arthritis: second revision, Edmonton, 2001. *J Rheumatol*. 2004;31(2):390–392.
 29. Berntson L, Fasth A, Andersson-Gäre B, et al. Construct validity of ILAR and EULAR criteria in juvenile idiopathic arthritis: a population based incidence study from the Nordic countries. International League of Associations for Rheumatology. European League Against Rheumatism. *J Rheumatol*. 2001;28(12):2737–2743.
 30. Cassidy J, Kivlin J, Lindsley C, Nocton J; Section on Rheumatology, Section on Ophthalmology. Ophthalmologic examinations in children with juvenile rheumatoid arthritis. *Pediatrics*. 2006;117(5):1843–1845.
 31. American Academy of Pediatrics, Section on Rheumatology and Section on Ophthalmology. Guidelines for ophthalmologic examinations in children with juvenile rheumatoid arthritis. *Pediatrics*. 1993;92:295–296.
 32. de Boer JH, Blankenberg-Sprenkels SHD, Erckens RJ, Los LI, Meenken C, Rothova A, van Velthoven MEJ, van Hagen PM, Armbrust W, Wulfraat NM, Dam-van Loon N, Fritsch-Stork RDE, de Groot-Mijnes JDF, Oosterheert JJ. 2015, <https://www.oogheekunde.org/sites/; www.oogheekunde.org/files/richtlijnen/Richtlijn-Uveitis-defgeautoriseerde-versie.pdf>.

33. Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One*. 2014;9(4):e95192.
34. Lundberg M, Eriksson A, Tran B, Assarsson E, Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res*. 2011;39(15):e102.
35. Kassambara A, Mundt F. factoextra: extract and visualize the results of multivariate data analyses. R package version 1.0.7. 2020.
36. Leek JT, Johnson WE, Parker HS, Fertig EJ, Jaffe AE, Zhang Y, Storey JD, Torres LC. sva: Surrogate Variable Analysis. R package version 3.38.0. 2020.
37. Liaw A, Wiener M. Classification and regression by randomForest. *R News*. 2002;2(3):18–22.
38. Kassambara A, Kosinski M, Biecek P. survminer: drawing survival curves using 'ggplot2'. R package version 0.4.9. 2021, <https://CRAN.R-project.org/package=survminer>.
39. Therneau T. A package for survival analysis in R. 2020. R package version 3.2-7, <https://CRAN.R-project.org/package=survival>.
40. Cann M, Ramanan AV, Crawford A, et al. Outcomes of non-infectious paediatric uveitis in the era of biologic therapy. *Pediatr Rheumatol Online J*. 2018;16(1):51.
41. Sardar E, Dusser P, Rousseau A, Bodaghi B, Labetoulle M, Koné-Paut I. Retrospective study evaluating treatment decisions and outcomes of childhood uveitis not associated with juvenile idiopathic arthritis. *J Pediatr*. 2017;186:131–137.e1.
42. West NR, Hegazy AN, Owens BMJ, et al. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease [published correction appears in *Nat Med*. 2017;23(6):788]. *Nat Med*. 2017;23(5):579–589.
43. Verhagen FH, Hiddingh S, Rijken R, et al. High-dimensional profiling reveals heterogeneity of the Th17 subset and its association with systemic immunomodulatory treatment in non-infectious uveitis. *Front Immunol*. 2018;9:2519.