Revising the Diagnosis of Idiopathic Uveitis by Peripheral Blood Transcriptomics

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• PURPOSE: To test the hypothesis that idiopathic uveitis can be categorized into subtypes based on gene expression from blood.

• DESIGN: Case control study.

• METHODS: We applied RNA-Seq to peripheral blood from patients with uveitis associated with 1 of 4 systemic diseases, including axial spondyloarthritis ($n = 17$), sarcoidosis ($n = 13$), inflammatory bowel disease ($n = 12$), tubulo-interstitial nephritis with uveitis ($n = 10$), or idiopathic uveitis ($n = 38$) as well as 18 healthy control subjects evaluated predominantly at Oregon Health and Science University. A high-dimensional negative binomial regression model implemented in the edgeR R package compared each disease group with the control subjects. The 20 most distinctive genes for each diagnosis were extracted. Of 80 genes, there were 75 unique genes. A classification algorithm was developed by fitting a gradient boosting tree with 5-fold cross-validation. Messenger RNA from subjects with idiopathic uveitis were analyzed to see if any fit clinically and by gene expression pattern with one of the diagnosable entities.

• RESULTS: For uveitis associated with a diagnosable systemic disease, gene expression profiling achieved an overall accuracy of 85% (balanced average of sensitivity plus specificity, $P < .001$). Although most patients with idiopathic uveitis presumably have none of these 4 associated systemic diseases, gene expression profiles helped to reclassify 11 of 38 subjects.

• CONCLUSIONS: Peripheral blood gene expression profiling is a potential adjunct in accurate differential diagnosis of the cause of uveitis. Validation of these results and characterization of the gene expression profile from additional discrete diagnoses could enhance the value of these observations. (Am J Ophthalmol 2021;222:15–23. © 2020 Elsevier Inc. All rights reserved.)

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INTRAOCULAR INFLAMMATION, ALSO KNOWN AS UVEitis, is a leading cause of acquired blindness.[1] Uveitis is frequently subdivided into broad categories, such as infections like syphilis or herpes simplex, immune-mediated disease confined to the eye, such as pars planitis or birdshot retinochoroidopathy, immune-mediated systemic diseases, such as sarcoidosis or ankylosing spondylitis, masquerade syndromes, such as lymphoma, and adverse reactions to medications, such as checkpoint inhibitors. Despite this broad differential diagnosis, the most common diagnosis from European or North American centers is usually idiopathic uveitis.[2,3] Other adjectives used to describe idiopathic uveitis include undifferentiated,[4] unclassifiable, or primary.[5] Each of these terms connotes a sense of uncertainty for both the clinician and the patient. The terms imply that the ophthalmologist does not understand the pathogenesis, and the terms do not provide information to guide either the patient or the physician regarding optimized therapy or prognosis. In contrast, classifying uveitis can provide invaluable information. For example, uveitis associated with Behçet disease, if untreated, often leads to blindness[6]; however, the use of tumor necrosis factor inhibitors has resulted in dramatically improved prognosis for this disease.[7,8] As another example, patients with sarcoidosis can have cardiac involvement, and therefore recognizing the ocular disease as the initial clinical manifestation of sarcoidosis can lead to potentially life-saving cardiac intervention.[9,10]

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Precision medicine is based on the concept that heterogeneous diseases like breast cancer or lymphoma can be subdivided based on a molecular characterization of the genes expressed in the malignant tissue.[11] We and others have attempted to apply this type of analysis to inflammatory diseases. For example, we can subdivide nonspecific orbital inflammation based on the genes expressed in the inflamed tissue.[12]

For uveitis, biopsy specimens of the affected uvea carry risk and are rarely obtained. However, the peripheral blood cells from patients with uveitis and a systemic illness can express a profile of gene transcripts that can aid in obtaining a specific diagnosis. We have previously described such a profile for patients with sarcoidosis[13] or ankylosing spondylitis.[14] In this report, we used RNA-Seq to characterize the gene expression profile in peripheral blood for 4 of the most common systemic, noninfectious diseases associated with uveitis: axial spondyloarthritis (AxSpA), inflammatory bowel disease (IBD), sarcoidosis, and tubulointerstitial nephritis with uveitis (TINU). While TINU is less common than the other 3 diagnoses, we[3] and others[15] have reported that it is a commonly missed cause of uveitis. We then characterized the peripheral blood gene expression from a group of patients with idiopathic uveitis to determine if we could arrive at a likely diagnosis on the basis of this transcriptional signature.

METHODS

• **STUDY DESIGN AND APPROVAL:** This case-control study compared gene expression levels in peripheral blood from subjects with idiopathic uveitis with levels from subjects with 1 of 4 defined forms of uveitis and from healthy control subjects. The study was conducted under approval by the Oregon Health and Science University (OHSU), Washington University, and the University of Utah institutional review boards as well as the central institutional review board at the University of Utah. All subjects provided written informed consent. Healthy control subjects were recruited from the comprehensive ophthalmology clinic at OHSU and had no history of uveitis or other systemic inflammatory disease by self-report or review of the medical record.

• **DIAGNOSTIC CRITERIA:** All patients with sarcoidosis had uveitis in association with symmetric hilar adenopathy, usually identified by computed tomography scans of the chest. This is consistent with criteria used in previous reports,[13] in which confirmation via a biopsy specimen was not required. Although granulomas on histology are characteristic of sarcoidosis, they are not definitive for diagnosis because granulomas can be caused by foreign bodies, fungal infection, or berylliosis. The combination of uveitis and hilar adenopathy to diagnose sarcoidosis is sometimes referred to as the Winterbauer criteria.[16] Patients with uveitis with AxSpA fulfilled the Assessment of SpondyloArthritis International Society criteria for this diagnosis, which allows for diagnosis in the absence of definitive radiographic disease based on multiple clinical features, such as inflammatory back pain, arthritis, uveitis, human leukocyte antigen–B27 positivity, elevated C-reactive protein, or other features of AxSpA.[17] Patients with uveitis with IBD were diagnosed by a gastroenterologist, usually on the basis of a biopsy specimen. They included 12 patients with ulcerative colitis and 4 patients with Crohn's disease. There are no absolute diagnostic criteria for TINU short of a renal biopsy specimen, which is not routinely performed on children in the United States. Patients with TINU in this series fulfilled characteristics as described by Mandeville and colleagues,[18] including typical uveitis, abnormal creatinine, abnormal urinalysis, and sometimes preceding systemic illness.

All subjects with uveitis were evaluated at the uveitis clinics of the Casey Eye Institute, OHSU, with the exception of 4 patients with TINU evaluated at either Washington University in St Louis, Missouri, or the Moran Eye Center in Salt Lake City, Utah. Patients with idiopathic uveitis did not fit into a diagnostic niche for uveitis as described previously.[3] In general, our diagnostic evaluation for patients with uveitis includes a comprehensive history. If no diagnosis is suggested by history, a serologic test for syphilis is ordered, usually a fluorescent treponemal antibody test. A chest radiograph is obtained. If the patient is >40 years of age, a computed tomography scan of the chest is requested if the patient and the patient's insurance permit this and the radiograph did not show adenopathy. Screening for tuberculosis is generally reserved for patients who have a risk factor for tuberculosis, such as being born outside of the United States.[19]

• **DATA MANAGEMENT:** All subject samples were given a unique identifier that was used in generating the RNA-Seq data. Subject data including personal identifiers and protected health information were linked to unique identifiers and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at OHSU.[20,21] REDCap is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

• **RNA-SEQ USING WHOLE BLOOD:** Our methodology has been described in detail.[22] Blood was collected in PAXgene Blood RNA Tubes (PreAnalytix/Qiagen, Zurich, Switzerland) and stored at –80 C. RNA was extracted using QIASymphony PAXgene Blood RNA kit (Qiagen, Hilden, Germany) according to the manufacturer's

TABLE 1. Demographics of Subjects

Subject Group	N	Average Age, Years (SD)	Female (%)
Axial spondyloarthritis	17	51.9 (12.9)	35.3
Inflammatory bowel disease	12	45.9 (18.6)	41.7
Tubulo-interstitial nephritis with uveitis	10	23.4 (14.9)	46.2
Sarcoidosis	13	69.9 (11.4)	68.4
Idiopathic uveitis	38	48.0 (20.6)	40.0
Healthy control subjects	18	42.8 (13.8)	61.1

SD = standard deviation.

instructions. A second DNase-treatment was performed followed by concentration and clean-up with the RNA Clean and Concentrator-96 kit (Zymo Research, Irvine, California, USA). Libraries were prepared from total RNA according to the manufacturer's instructions using the TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold (Illumina). Libraries were pooled at 3 samples per lane and sequenced using a single read, 100 cycle protocol on a HiSeq 2500 (Illumina, Madison, Wisconsin, USA). The quality of the raw sequencing files was evaluated using FastQC[23] combined with MultiQC.[24,25] Trimmomatic[26] was used to remove any remaining Illumina adapters. Reads were aligned to Ensembl's GRCh38 human reference genome along with its corresponding annotation, release 98. The program STAR[27] (version 2.7.3a) was used to align the reads to the genome. STAR has been shown to perform well compared with other RNA-seq aligners.[28] Because STAR uses the gene annotation file, it also calculated the number of reads aligned to each gene. RNA-SeQC[29] and another round of MultiQC were used to ensure alignments were of sufficient quality.

• **STATISTICS:** Uniquely aligned gene counts were imported into R statistical language[30] for statistical analyses. The counts were normalized by the trimmed mean of M-values (TMM)[31] and filtered for genes with low counts by the filterByExpr function in the edgeR[32] package for R. The differential gene expression analysis was performed by the negative binomial regression models implemented in the edgeR R package and the removing unwanted variation methods by Risso and associates[33] available in the RUVseq R package. The gradient boosting tree was fitted by the gbm function of the gbm R package.[34] Five-fold cross-validation was used to avoid potential overfitting.

RESULTS

TABLE 1 SHOWS THE AGE AND SEX DISTRIBUTIONS FOR THE subjects in this study. As expected, patients with TINU are on average younger than the patients from the other categories of uveitis. Patients with AxSpA were more likely to be male. The statistical methods used helped exclude bias on the basis of age or sex.

A negative binomial regression model with removing unwanted variation methods was used to identify significantly differentially expressed genes in each disease group relative to healthy control subjects. From the fitted results, the top 20 significant genes based on the false discovery rate *P* values were extracted. Table 2 lists the top 20 genes that were most distinctive for each diagnosis with fold changes and false discover rate *P* values. There were 75 unique transcripts among them. From these 75 unique genes, a classification model was developed by the gradient boosting tree algorithm.[35] A classification model is a statistical term that should not be confused with classification criteria for a given disease, a concept explained in the discussion below. The gradient boosting algorithm is one of the most popular supervised learning methods. When developing a classification rule using a supervised machine learning method, one of the common concerns is overfitting, which can lead to a model that will be less generalizable and have poor prediction performance for future observations. Indeed, we were able to achieve zero training errors (ie, 100% accuracy) while the gbm used 63 genes among the 75 genes. However, to prevent overfitting, 5-fold cross-validation was used, which provides more realistic prediction errors in general. The algorithm used only 21 of the 75 genes. Figure 1, A shows a 3-dimensional plot based on the principal component analysis of the 21 genes for the 4 prototypical uveitic diagnoses. The figure indicates that reasonable separation can be achieved based on the gene expression for these 4 entities. Figure 1, B adds the patients with idiopathic uveitis to this plot. The relative importance of specific transcripts is shown in Figure 2. Table 3 shows the sensitivity, specificity, and balanced accuracy for each of these 4 diagnoses based on gene expression profiling from 5-fold cross-validation.

To assess the diagnostic utility of this algorithm, we calculated a diagnostic likelihood for each of the 4 diagnoses for the 38 patients with idiopathic uveitis. We then reviewed the history and ophthalmic examination findings to see how each fit with the likely diagnosis. In 11 instances, we concluded that the gene expression profile helped identify a probable diagnosis. Table 4 describes these 11 subjects in more detail. Table 4 relies in part on the consistent phenotype of uveitis that is characteristic of ankylosing spondylitis,[36] the relatively frequent finding of disc edema accompanying the bilateral anterior uveitis of TINU,[37] and the >50% likelihood that a female ≥61 years of age with idiopathic uveitis will have sarcoidosis.[38] In addition, 1 subject had microscopic colitis and retinal vasculitis. Microscopic colitis is a rare variant of inflammatory bowel disease and we presumed that it was causally related to her eye disease. However, the gene expression profile of this subject did not suggest IBD as the leading

TABLE 2. Top 20 Significantly Differentially Expressed Genes in Each Disease Group Against Healthy Control Subjects

Axial Spondyloarthritis			Inflammatory Bowel Disease			Tubulo-Interstitial Nephritis with Uveitis			Sarcoidosis		
Symbol	Fold Change[a]	FDR *P* Value	Symbol	Fold Change[a]	FDR *P* Value	Symbol	Fold Change[a]	FDR *P* Value	Symbol	Fold Change[a]	FDR *P* Value
SLC41A1	−1.2	<.0001	LINC02210	−1.4	<.0001	MOB3A	1.4	<.0001	CAND1	−1.3	<.0001
CASP5	1.9	<.0001	FOLR3	2.9	<.0001	AC118549.1	−1.4	<.0001	SACS	−1.5	<.0001
CASP4	1.3	.0002	DDX3Y	58.7	<.0001	PRPF39	−1.4	<.0001	DYRK2	−1.5	<.0001
LINC02210	−1.3	.0002	KDM5D	70.9	<.0001	FUBP1	−1.3	<.0001	LINC02210	−1.6	<.0001
RPL10P6	−33.0	.0003	RPS4Y1	37.0	<.0001	MARK2	1.3	<.0001	SLC38A1	−1.5	<.0001
ACSL4	1.2	.0004	AC087672.2	1.9	<.0001	LASP1	1.3	<.0001	ZNF780B	−1.4	<.0001
REPIN1	−1.2	.0004	PRKY	41.0	<.0001	PLEKHM1	1.3	<.0001	PLEKHA1	−1.5	<.0001
RPL10P9	−20.8	.0004	LINC00278	61.0	<.0001	ARMC1	−1.3	<.0001	SLC41A1	−1.4	<.0001
YBX1P4	−1.8	.0005	LINC02649	1.7	<.0001	URI1	−1.4	<.0001	AC107027.3	−1.6	<.0001
TLR4	1.2	.0006	TTTY14	22.6	<.0001	NAA15	−1.3	<.0001	ZNF549	−1.4	<.0001
CABIN1	−1.2	.0006	C3orf86	1.5	<.0001	SHPRH	−1.3	<.0001	DENND11	−1.4	<.0001
USP15	1.1	.0011	ZFY	25.4	<.0001	GMIP	1.4	<.0001	AC118549.1	−1.3	<.0001
OSTF1	1.1	.0011	USP9Y	44.4	<.0001	CAND1	−1.3	<.0001	ZNF510	−1.4	<.0001
CA8	−2.1	.0011	ANXA3	2.1	<.0001	SYNJ2BP	−1.4	<.0001	GTPBP8	−1.3	<.0001
TTC26	1.8	.0012	TXLNGY	67.5	<.0001	PXN	1.4	<.0001	ZFP14	−1.4	<.0001
MTA1	−1.1	.0012	IFI27	7.4	<.0001	NOL11	−1.3	<.0001	ETS1	−1.5	<.0001
USP8	1.1	.0013	BCORP1	16.4	<.0001	SYNCRIP	−1.3	<.0001	GDF11	−1.6	<.0001
SPECC1L	−1.2	.0013	B2M	1.3	<.0001	ARRB2	1.4	<.0001	OXCT1	−1.4	<.0001
AC004797.1	−1.5	.0014	ALOX5	1.2	<.0001	GOLGA8B	−1.6	<.0001	SFXN1	−1.5	<.0001
FMR1	1.1	.0014	PFKFB3	1.5	<.0001	U2SURP	−1.3	<.0001	BCL11B	−1.5	<.0001

FDR = false discovery rate.

Transcripts vary widely with regard to fold change. Transcripts were chosen on the basis of FDR *P* values rather than on the basis of fold change so one should not conclude that certain diseases are characterized by a greater fold change.

[a]Negative fold change indicates a down-regulated gene in the disease group relative to the healthy control subjects.

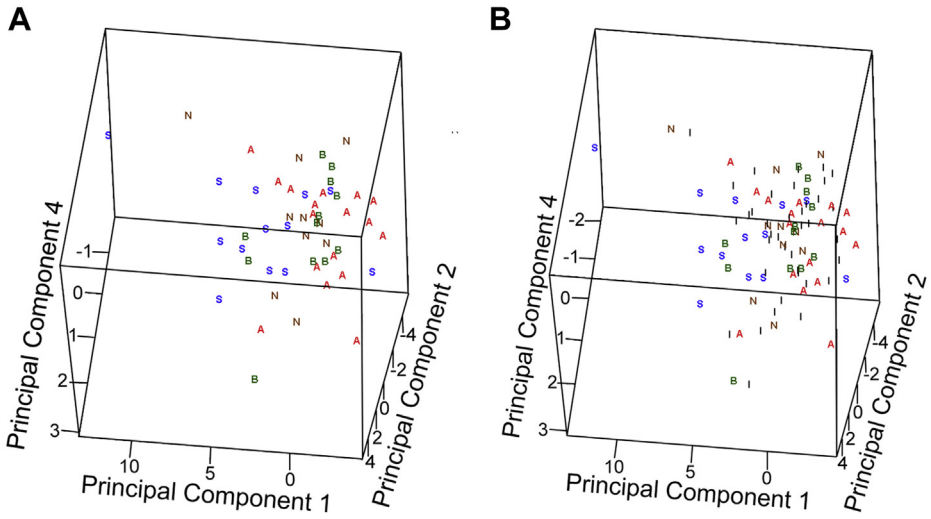

FIGURE 1. Principal component plots. Principal components in 3 dimensions are plotted for (A) the 4 known uveitis disease groups and (B) the idiopathic uveitis subjects are shown among the 4 known uveitis disease groups. The principal component analysis was based on the 21 most important genes for prototypical uveitis diagnoses as shown in Table 2. Letters in the plots refer to each disease group: A, axial spondyloarthritis; B, inflammatory bowel disease; I, idiopathic uveitis; N, tubulo-interstitial nephritis with uveitis; S, sarcoidosis.

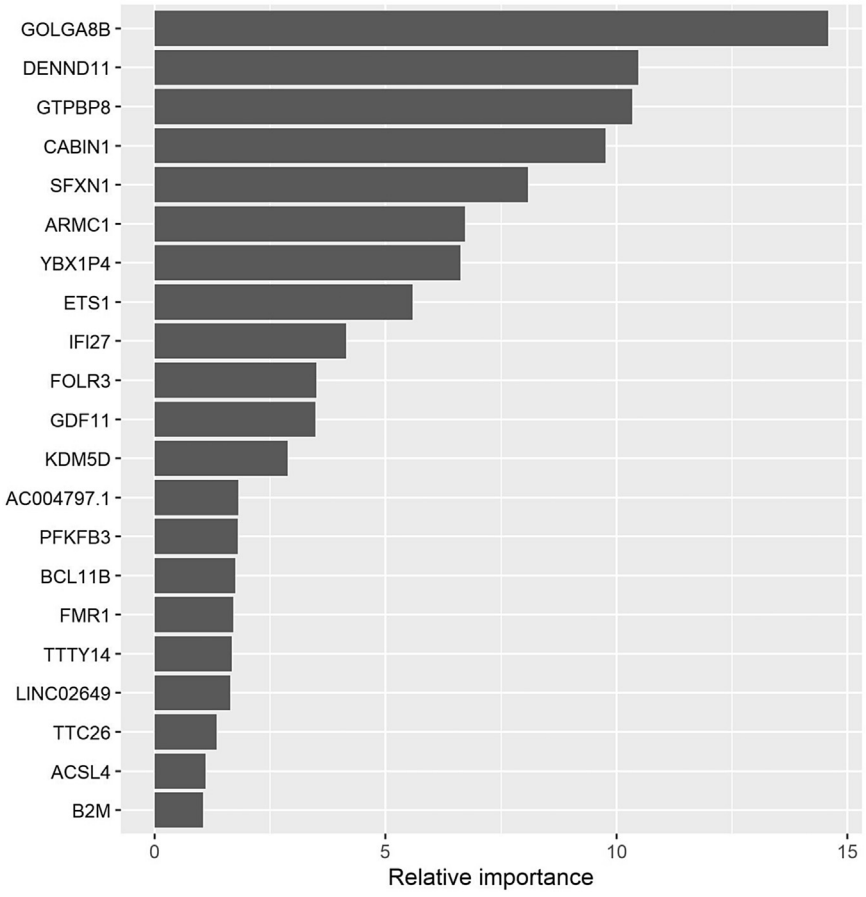

FIGURE 2. Relative importance of the 21 predictive genes. The 21 genes used in the diagnostic algorithm are arranged according to their relative importance for classification of 4 disease groups.

TABLE 3. Estimated Testing Accuracy of Each Known Uveitis Subgroup by Gradient Boosting Tree Classification with 5-fold Cross-Validation

	Axial Spondyloarthritis	Inflammatory Bowel Disease	Tubulo-Interstitial Nephritis with Uveitis	Sarcoidosis
Sensitivity	88%	67%	60%	92%
Specificity	91%	98%	100%	82%
Balanced accuracy	90%	82%	80%	87%

diagnostic possibility and her retinal vasculitis remains idiopathic. A recent clinical review of microscopic or collagenous colitis also concluded that it was not associated with eye disease.[39]

DISCUSSION

IN THIS STUDY, WE SHOW THAT AN ANALYSIS OF GENE expression from peripheral blood has the potential to aid in the diagnosis of uveitis. Physicians who classify disease are sometimes nicknamed "lumpers" or "splitters." The rationale to split is far greater if subdividing has implications for the choice of therapy or prognostic implications. If a biomarker supports the subcategorization, the case for subdividing is similarly strengthened. Clearly, "idiopathic" disease is an ideal diagnosis to be tackled by splitters because the term connotes heterogeneity and uncertainty.

The differential diagnosis of uveitis includes dozens of subcategories. We chose to focus on 4 because in our previous efforts to categorize uveitis these were the 4 diagnoses most commonly encountered and most often missed in the United States by referring community ophthalmologists.[3] If we had performed a molecular categorization for Behçet disease, juvenile idiopathic arthritis, pars planitis,

TABLE 4. Suggested Uveitis Revised Diagnosis of Idiopathic Uveitis Based on Gene Expression Profiling

Age (y)	Sex	Uveitis Phenotype	Systemic Symptoms or Findings	Predicted Diagnosis[a]	Additional Clinical Comments Based on Postprediction Chart Review
28	M	Unilateral AAU with vascular sheathing	HLA-B27 negative	Sarcoidosis 37%	Consistent with retinal vasculitis
40	F	Recurrent, unilateral AAU	HLA-B27 negative	AxSpA 34%	Uveitis phenotype fits AS[34]
15	F	Bilateral, chronic anterior uveitis	Sterile pyuria	TINU 34%	Age, uveitis, urine consistent with TINU
84	F	Bilateral chronic panuveitis	None	Sarcoidosis 45%	Chorioretinal scarring suggestive of sarcoidosis; negative chest CT scan but >50% of females with idiopathic uveitis >61 years of age have been found to have sarcoidosis[36]
58	F	Bilateral, chronic anterior and intermediate uveitis	Transverse colon biopsy specimen consistent with Crohn's disease	IBD 31%	Minimal bowel symptoms; biopsy confirmation several years after uveitis onset
37	F	Recurrent unilateral AAU	Diarrhea and joint pain	IBD 33% and AxSpA 32%	Uveitis fits either IBD or AS
39	F	Bilateral, chronic anterior uveitis with disc edema	None	TINU 27%	TINU considered in initial differential; presence of disc edema with anterior uveitis well described in TINU[35]
79	F	Recurrent, unilateral anterior and intermediate uveitis	Right upper lobe nodule	Sarcoidosis 32%	Chest CT scan was negative, age consistent with sarcoidosis
44	M	Recurrent, unilateral AAU	HLA-B27 positive	AxSpA 57%	Experienced subsequent back pain two years after enrollment with normal sacroiliac joint X-rays as is expected with non-radiographic AxSpA
56	M	Bilateral panuveitis	Systemic onset weight loss and calcified hilar notes	IBD 33% and sarcoidosis 32%	Consistent with sarcoidosis, especially with systemic onset and calcified nodes found on chest CT scan 15 months after initial uveitis evaluation
39	M	Bilateral chronic anterior uveitis with retinal vasculitis	Plantar fasciitis and joint pain	AxSpA 37%	Uveitis not suggestive of AS but heel pain is typical[35]; cannot exclude uveitis as separate from arthritis/heel pain

AAU = acute anterior uveitis; AS = ankylosing spondylitis; AxSpA = axial spondyloarthritis; CT = computed tomography; F = female; HLA = human leukocyte antigen; IBD = inflammatory bowel disease; M = male; TINU = tubulo-interstitial nephritis with uveitis.

[a]Percent of diagnostic likelihood (see text for explanation).

tuberculosis, etc, it is possible that we would have succeeded in assigning labels for more of the idiopathic subjects.

Certainly, some patients with idiopathic uveitis might have a disease with a unifying pathogenesis, course, or response to therapy and be distinguishable from all current known subsets of uveitis. In other words, we presently have no way to know how many among the 38 idiopathic subjects could be potentially classified into a known entity and how many should be classified into a novel entity. We are in the process of evaluating gene expression in idiopathic uveitis to determine if this diagnosis includes distinct subsets that are not currently recognized in our diagnostic rubric.

Most uveitis experts have encountered patients with uveitis characteristic of juvenile idiopathic arthritis (insidious onset during early childhood, bilateral, chronic duration) but in whom no arthritis was present. Similarly, uveitis centers evaluate patients whose uveitis resembles Behçet disease (sudden onset, episodic with incomplete clearing between attacks, bilateral, panuveitis with retinal vasculitis) but have no other hallmark of Behçet disease, such as oral or genital ulcers. In examples like these, traditional laboratory evaluations (antinuclear antibodies, human leukocyte antigen–B51) are not specific, but a gene expression platform could help establish the diagnosis and would certainly have therapeutic and prognostic implications.

Our study includes advantages by focusing on whole blood rather than isolated populations or ocular cells: 1) obtaining ocular samples poses risk and inconvenience and is limited to sampling patients with active disease; 2) it avoids alteration of data by experimental manipulation of samples and cells; and 3) it was not limited to gene expression signatures provided by a single cell type but allows unbiased discovery of genes expressed by multiple cell types. In designing this study, we encountered several decision points. For example, we could have studied mononuclear cells from the eye. We were dissuaded from this approach because of the need for an invasive procedure to obtain these cells and because the small sampling would likely be greatly affected by disease duration and treatment. We could have isolated a subpopulation of cells such as CD4 T lymphocytes from peripheral blood. We opted not to do this because it would add to the complexity of reproducing our studies and because the process of isolation could lead to some artefactual activation. Studying gene expression from peripheral blood entails the challenge that globin gene expression could obscure the expression of genes from leukocytes. We addressed this by performing extensive preliminary studies to assess the impact of globin gene expression on RNA-Seq and the pros and cons of removing globin gene transcripts before library preparation.[22] Single-cell RNA-Seq is an exciting, emerging technology that could yield an additional modality to characterize the pathogenesis of uveitis. This will be an important adjunct that is highly deserving of further study as an approach to understand eye inflammation.

Our study should be considered a pilot study that supports a proof of principle. Studies that involve multiple statistical comparisons should include a discovery and a validation set. We have some validation because we have previously characterized the peripheral blood gene expression in both ankylosing spondylitis[14] and sarcoidosis,[13] but we have not validated our findings for IBD or TINU. We recognize that factors such as age, sex, disease duration, medications, geographic location, and comorbidities can affect results. Our data set is too small to make conclusions about most of these variables. We did not distinguish between Crohn's disease and ulcerative colitis because of a limited number of subjects with IBD. Such a distinction could result in a greater ability to subclassify patients with uveitis. Despite these limitations, we hope that our observations will inspire additional work to refine and extend our observations. Classification criteria exist for many diseases. Classification criteria promote specificity over sensitivity so that a uniform population can be studied as in a clinical trial.[40] The revised diagnosis that we illustrate in Table 4 is meant to reflect the process of differential diagnosis which usually favors sensitivity over specificity in contrast to classification. The absence of diagnostic criteria to validate the probable diagnosis as in Table 4 is a weakness of this study.

In summary, idiopathic uveitis remains a daunting challenge, but refinements in RNA sequencing are leading to novel approaches to identify specific subsets among patients with idiopathic uveitis.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

JAMES T. ROSENBAUM: CONCEPTUALIZATION, FORMAL analysis, Funding acquisition, Investigation, Writing - original draft, Writing - review & editing. **Christina A. Harrington:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing - review & editing. **Robert P. Searles:** Data curation, Formal analysis, Writing - review & editing. **Suzanne S. Fei:** Data curation, Formal analysis, Writing - review & editing. **Amr Zaki:** Investigation, Writing - review & editing. **Sruthi Arepalli:** Investigation, Writing - review & editing. **Michael A. Paley:** Investigation, Writing - review & editing. **Lynn M. Hassman:** Investigation, Writing - review & editing. **Albert T. Vitale:** Investigation, Writing - review & editing. **Christopher D. Conrady:** Investigation, Writing - review & editing. **Puthyda Keath:** Investigation, Writing - review &

editing. **Claire Mitchell:** Investigation, Writing - review & editing. **Lindsey Watson:** Investigation, Writing - review & editing. **Stephen R. Planck:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Writing - review & editing. **Tammy M. Martin:** Data curation, Formal analysis, Writing - review & editing. **Dongseok Choi:** Conceptualization, Data curation, Formal analysis, Writing - review & editing, Funding acquisition.

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