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# Autoantibody profiling in intraocular fluid of patients with uveitis

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ABSTRACT

A high prevalence of serum antiretinal antibodies (ARAs) in patients with uveitis has been previously described, though their clinical role remains elusive. Assessment of intraocular ARAs may provide further insight into the pathogenesis of diverse uveitis entities. In this study we investigate the prevalence of multiple specific anti-ocular antibodies (AOcAs), including ARAs, in intraocular fluid of patients with uveitis. Autoantibody profiling with 188 different ocular antigens was performed by a multiplex immunoassay with intraocular fluid samples of 76 patients with uveitis. Clinical data from uveitis patients were collected and statistical analyses were executed to evaluate associations between intraocular AOcAs and clinical characteristics. Controls consisted of 19 intraocular fluid samples from cataract patients. A spectrum of 22 different AOcAs was present in higher levels in patients with uveitis than in controls ($p < 0.05$), but in moderately elevated titers (< 2x). High elevations of intraocular AOcAs in uveitis (> 5x compared to cataract) were observed in varicella zoster virus-induced uveitis, multiple sclerosis-associated uveitis and patients with unexplained uveitis but positive quantiferon test. Presence of macular edema was associated with increased intraocular levels of tyrosinase antibodies. Our results show that patients with uveitis are characterized by the presence of a broad spectrum of moderately elevated levels of intraocular AOcAs, and high intraocular AOcA levels were found in several specific uveitis entities. This study favors secondary production of AOcAs and not their inciting role.

## 1. Introduction

Uveitis is a severe ocular disease, which can result in permanent blindness. Uveitis has multiple causes including infections and shows strong associations with various systemic immune-mediated diseases. The pathogenesis of most uveitis entities is not fully understood, but the immune system plays a crucial role; especially the development of autoimmune intraocular reactions in non-infectious uveitis has been repeatedly proposed, but scarcely proven. Serum antibodies directed against retinal tissue were more prevalent in patients with uveitis compared to healthy controls(Ten Berge et al., 2016a). It has been hypothesized that the antiretinal antibodies (ARAs) in uveitis might either incite the ocular disease or represent a secondary epiphenomenon induced by retinal damage. The possible pathogenic role of these ARAs is unknown, although it has been suggested that ARAs might aggravate and/or prolong the ocular disease(Chant et al., 1985; Heckenlively et al., 1996; Ten Berge et al., 2017).

The eye is an immune privileged organ and intraocular ARAs might show an entirely different profile than ARAs found in the peripheral blood. This phenomenon has been shown previously in infectious uveitis, in which local production of specific antibodies is regarded as indirect proof of the intraocular infection(Rothova et al., 2008). Similarly, in neurological diseases including autoimmune encephalitis and multiple sclerosis the importance of analysis of cerebrospinal fluid has been previously proven(Dalmau et al., 2011) (Quintana et al., 2012). Currently, antigen bead arrays are successfully being used for the analysis of autoantibodies. This technique enables analysis of very small volumes (such as intraocular fluids) towards hundreds of different antigens and its potential for ARA detection has been suggested(Bazhin, 2009).

Herein, we perform an autoantibody profiling of intraocular fluid samples of patients with diverse uveitis entities and assess the prevalence of antibodies directed against 188 different ocular antigens as potential targets, and relate the results to clinical manifestations of uveitis.

## 2. Methods

Remainders of diagnostic intraocular fluid samples from 76 patients with uveitis were collected from the Laboratory of Virology of the

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Erasmus MC, University Medical Center Rotterdam between February 2009 and April 2015. Intraocular fluid samples of 19 patients with cataract stored in the biobank in the same time period were used as controls. All intraocular fluid samples were stored at −80 °C. This study was approved by the local ethical committee from the Erasmus University Medical Center (Medical Ethics Committee Erasmus MC) and adhered to the tenets of the Declaration of Helsinki.

All uveitis patients were classified according to the localization of uveitis using the Standardization of Uveitis Nomenclature SUN criteria (Jabs et al., 2005; Trusko et al., 2013). Patients underwent a standardized diagnostic protocol based on this anatomical site of inflammation. The protocol included chest radiography, erythrocyte sedimentation rate, blood counts, serum angiotensin-converting enzyme levels, serology for syphilis and Lyme disease and interferon gamma release assay (IGRA) test (QuantiFERON–TB Gold In-Tube test). In patients with anterior uveitis or panuveitis Human Leukocyte Antigen (HLA)-B27 testing was also performed. According to the clinical uveitis manifestations, further examinations were added (tailored approach). Specific diagnoses were determined after the various diagnostic procedures were completed. The diagnosis of intraocular infections was always confirmed by polymerase chain reaction (PCR) and/or Goldmann-Wittmer coefficient in intraocular fluid. The diagnosis of Fuchs uveitis syndrome (FUS) was based on clinical characteristics. The diagnosis of sarcoidosis was either histologically proven or based on chest imaging in patients with otherwise unexplained uveitis. All other specific diagnoses were made according to current diagnostic criteria(Arcinue et al., 2013; Arnett et al., 1988; Minos et al., 2016).

Clinical data of patients with uveitis were collected from medical data files. We registered age, gender, location of uveitis and specific cause of uveitis. Further, we registered the following characteristics at the moment of sample collection: duration of uveitis, presence of cystoid macular edema (CME), presence of vasculitis (determined either by funduscopic examination or by fluorescence angiography), use of systemic immunosuppressive medication and activity of uveitis (presence of cells in the anterior chamber and/or vitreous, keratic precipitates, choroiditis, retinitis, vasculitis and/or papillitis)(Deschenes et al., 2008).

Autoantibody profiling with 188 different ocular antigens, representing 97 unique ocular proteins, was performed on intraocular fluid samples from patients with uveitis (N = 76) and cataract (N = 19; supplementary table 1). Antigens were selected based on potential relevance to ocular diseases according to literature or previous retinal immunohistochemistry staining. The used antigens were protein fragments produced within the Human Protein Atlas, designed to have low homology to other human proteins and expressed in *Escherichia coli* with an affinity tag consisting of six histidines and an albumin-binding domain from streptococcal protein G ($His_6$ABP)(Persson et al., 2006; Uhlen et al., 2005). A multiplex assay, previously validated by immunoblot and immunohistochemistry, was performed as described before with minor alterations(Ten Berge et al., 2016b). In short, the antigens were covalently coupled to color-coded magnetic beads to create a bead array. The samples were diluted 1:10 in assay buffer (0.1% PBS-Tween20, 3% BSA, 160 μg/ml $His_6$ABP), let to pre-block potential antibodies towards the ABP-domain for 1 h in room temperature, and subsequently incubated O/N in room temperature with the bead array. Interactions were fixated with 0.2% PFA for 10 min before incubation for 30 min with a fluorophore conjugated anti-human IgG Fab fragment. A FlexMap3D instrument (Luminex Corp.) was used to acquire a read-out. The autoantibody profiling was performed at the SciLifeLab Autoimmunity Profiling Facility in Stockholm, Sweden.

For the statistical analyses, continuous variables were summarized using medians and ranges, and categorical variables were summarized using percentages. Patient demographics were compared between diagnosis groups using Mann Whitney U tests for continuous data and Fisher's exact tests for categorical data. Linear regressions with correction for age and gender were performed to analyse differences between the diagnosis groups (uveitis or specific uveitis entities versus cataract). In addition subgroup analyses of these linear regressions were performed to compare all specific uveitis entities with cataract. Some specific uveitis entities were not analysed as separate groups (uveitis associated with herpes simplex virus (HSV), Sjögren syndrome, HLA B27-associated uveitis and birdshot chorioretinopathy), because their numbers were too small. To analyse differences within the uveitis group we performed linear regressions with age, gender, location of uveitis (reference category: anterior uveitis), uveitis entity (reference category: uveitis associated to sarcoidosis), uveitis activity, systemic immunosuppressive therapy, presence of CME and presence of vasculitis as independent variables. All regression analyses were performed using the natural logarithm of the measured median fluorescent intensities as dependent variable. Relative (fold) increases of differences in levels of AOcAs were calculated by exponentiating the estimated regression coefficients. The linear regressions were performed with a robust MM-type estimation method, to account for the fact that the model residuals were not normally distributed(Koller and Stahel, 2011). We used the signal intensities as an indication for the levels of intraocular AOcAs. To adjust for the multiple comparisons of the different antigens, a Bonferroni correction was applied (all p-values were multiplied by 188). P-values of $< 0.05$ were considered statistically significant. All statistical tests were two-sided. The analyses were performed using SPSS and R (version 3.3.1), with the robustbase package for the robust linear regressions(Team, 2013).

## 3. Results

The median age of the 76 included uveitis patients was 48 years, and 46% of patients were males. Median duration of uveitis was two years (ranging from zero to 36 years), and 51 (67%) cases were active during sample collection. Patients' characteristics are shown in Table 1. The most prevalent cause of uveitis in our cohort was sarcoidosis (14/76, 18%). Nine patients with sarcoidosis-associated uveitis were biopsy proven and in five patients the diagnosis was based on radiologic

**Table 1**
Characteristics of uveitis patients (N = 76).

| Characteristic | Number (%) |
|---|---|
| **Male-to-female ratio** | 35 - 41 (46%–54%) |
| **Median age in years (range)** | 48 (17–86) |
| **Median duration uveitis in years (range)** | 2 (0–36) |
| **Location** | |
| Anterior | 26 (34%) |
| Intermediate | 10 (13%) |
| Posterior | 23 (30%) |
| Panuveitis | 17 (22%) |
| **Specific cause or association** | |
| *Infectious* | 36 (47%) |
| Rubella | 12 (16%) |
| Toxoplasma gondii | 8 (11%) |
| Varicella zoster virus | 7 (9%) |
| Cytomegalovirus | 7 (9%) |
| Herpes simplex virus | 2 (3%) |
| *Systemic disease* | 24 (32%) |
| Sarcoidosis | 14 (18%) |
| Multiple sclerosis | 8 (11%) |
| Sjögren syndrome | 1 (1%) |
| HLA B27 + | 1 (1%) |
| Ocular lymphoma | 5 (7%) |
| *Clinical entity* | 3 (4%) |
| Birdshot chorioretinopathy | 2 (3%) |
| Fuchs uveitis syndrome | 1 (1%) |
| *Unknown* | 8 (11%) |
| Quantiferon positive | 6 (8%) |
| **Active uveitis** | 51 (67%) |
| **Presence of cystoid macular edema** | 19 (25%) |
| **Presence of vasculitis** | 16 (21%) |
| **Systemic immunosuppressive treatment** | 16 (21%) |