Diagnosing Uveitis: Value and Limitations of Current Diagnostic Tests

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The studies presented in this thesis were conducted at the Department of Ophthalmology and the Department of Immunology, Erasmus MC, University Medical Center Rotterdam, the Netherlands.

The work described in this thesis was financially supported by AbbVie Nederland.

The printing of this thesis was financially supported by Rotterdamse Stichting Blindenbelangen, Landelijke Stichting voor Blinden en Slechtzienden and Stichting Blindenhulp.

Cover design and layout: © evelienjagtman.com
Printed by: Gildeprint
ISBN: 9789463234955

Diagnosing Uveitis: Value and Limitations of Current Diagnostic Tests

Diagnosticeren van Uveitis: de Waarde en Beperkingen van Huidige Diagnostische Testen

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus

prof.dr. R.F.M.E. Engels

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

Woensdag 10 April 2019 om 9.30 uur

Fahriye Hakan-Groen
geboren te Berlijn, Duitsland

Erasmus University Rotterdam

Promotiecommissie

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chapter 1

General Introduction and Aims of this Thesis

General Introduction

Uveitis

Uveitis means inflammation of the uvea, the middle vascular layer of the eyeball. It affects mostly the working age group and causes 10-15% of preventable blindness in Western countries.[1-4] In everyday practice, the term uveitis is principally used as an umbrella for all types of intraocular inflammation. Originally this expression was used for the first time approximately 200 years ago and is derived from the Latin *uva* (or grape).[5] The term uvea was given by anatomists, who found that the uvea resembled the appearance of grapes after they were peeled. In this general introduction, the causes, classification and current diagnostic work-up of uveitis will be discussed.

Causes of uveitis over time

The opinion of ophthalmologists on the causes of uveitis changed through time. In the past, ophthalmologists focused predominantly on two infectious causes of uveitis. 'Any form of uveitis should alert the clinician to the possibility of tuberculosis or syphilis', a quote from an uveitis manual by Smith and Nozik, reflects the simple differential diagnosis of uveitis in the middle of the 19th century.[6-10] However, the number of uveitis cases attributed to syphilis decreased with the introduction of the Wassermann reaction, an antibody test for syphilis, developed in 1906 and the introduction of penicillin treatment after its discovery in 1928.[8] In the later part of that century uveitis was occasionally attributed to localized infections elsewhere in the body and many teeth were extracted in an attempt to treat uveitis.[8]

In the beginning of the 20th century, the concept of autoimmune responses as a possible cause of uveitis emerged.[11,12] Autoimmunity was suspected in many uveitis cases, but was only proven in a minority of patients (e.g. multiple sclerosis, Vogt-Koyanagi-Harada disease, granulomatosis with polyangiitis).[13-15]

In recent years, the concept of immunological diseases was re-defined and the model of autoimmune and autoinflammatory diseases emerged. Originally the term autoinflammatory was introduced in 1999 to denote patients with hereditary periodic fever syndromes.[16] More recently, these immunological diseases were proposed to be re-classified into autoimmune, autoinflammatory and mixed autoimmune/ autoinflammatory diseases.[17-22] Simply said, autoimmunity is self-directed inflammation where autoreactive B- and T-cell responses and autoantibodies are central; in contrast to autoinflammatory disease. In the latter involvement of the innate immune system characterized by inappropriate activation of the inflammasome resulting in exaggerated release of interleukin (IL)-1beta causes inflammatory symptoms.[20,23] Sarcoidosis, first reported to occur in the eye in 1914, is probably the most common autoinflammatory disorder in the uveitis population.[24-27]

More emphasis was put on various possible infectious causes of uveitis in the late 20th century, when the novel molecular and serologic diagnostic tests, adapted for small volumes, were introduced. Various parasitic and viral causes of uveitis were discovered and are still discovered. The differential diagnosis of infectious causes of uveitis expanded, placing more emphasis on *Toxoplasma gondii* and viral agents. In addition to common and widely recognized viral causes of uveitis such as Herpes Simplex Virus, Varicella Zoster Virus and Cytomegalovirus, Rubella virus was associated to uveitis and linked to Fuchs Uveitis Syndrome in 2004.[28,29]. The oncogenic human pathogen Human T-cell lymphotropic virus type 1 (HTLV-1), causing adult T cell leukemia-lymphoma (ATL) and HTLV-1-associated myelopathy (HAM) was linked to uveitis in 1989 for the first time in Japan.[30] One report of seropositive HTLV-1 patients among patients with HAM and seropositive patients without neurologic symptoms showed uveitis prevalence of around 14% among both groups, higher than the proportion of uveitis in the general population.[31] Chikungunya virus was first linked to uveitis in 2007 and causes mainly non-granulomatous anterior uveitis.[32-34] Several viral agents were discovered during more recent epidemics. Survivors of Ebola virus may suffer from uveitis after systemic recovery from the disease in around 14% and the first evidence of the virus in ocular fluid was substantiated in 2015.[35] Several reports have described the ocular complications of Zika virus in adults during acute infection, including iridocyclitis and retinitis.[36]

Also, uveitis as a manifestation of disorders related to HLA antigens became recognized, such as HLA B27- associated uveitis, birdshot chorioretinopathy (BSCR; which is associated to HLA A29) and Behçet's disease (associated to HLA B51).

Nowadays, it is recognized that in 40-60% of uveitis cases an underlying systemic disease is identified (infectious or noninfectious).[37] Since 2008 the etiology of uveitis is being categorized in 3 major groups according to the International Uveitis Study Group (IUSG, Table 1). These categories include infectious uveitis, non-infectious uveitis and masquerade syndromes.[38] Noninfectious uveitis without any associated systemic disease compromise also a spectrum of recognized ocular syndromes, while the pathogenesis in these entities remains mostly unknown. This classification of etiology was aimed to help in the evaluation and diagnosis of uveitis and is now widely used.

Classifications of uveitis

In the majority of patients presenting with uveitis for the first time, the cause is not clear. Even after a diagnostic work-up, the underlying cause remains unknown in a substantial proportion of patients.[37] Therefore, the physical appearance of an inflamed eye requires proper classification in order to communicate in clinical and research settings.

One of the first classification systems was the subdivision into granulomatous (with usually a chronic course, large keratic precipitates (KPs) and sometimes visible granulomas) and non-granulomatous uveitis (usually more acute without large KP's). However, these descriptions are vague and do not correlate with histopathologic findings and moreover, the aspect of KPs may change during the course of disease.

TABLE 1. Causes of uveitis.

Infectious	**Bacterial**	- Bartonella henselae, Borrelia burgdorferi, Brucella melitensis and Brucella abortus - Cutibacterium (Propionibacterium) - Leptospira - Mycobacterium tuberculosis, Mycobacterium leprae and atypical Mycobacteria - Rickettsia rickettsii - Treponema pallidum, Tropheryma whippelii
	Viral	- Chikungunya virus, Cytomegalovirus - Dengue virus - Ebola virus - Herpes simplex virus, Human Immunodeficiency virus type 1, Human T-cell lymphotropic virus type 1 - Measles virus, Mumps virus - Rubella virus - Varicella Zoster Virus, Vaccinia virus - West Nile virus - Zika virus
	Fungal	- Aspergillus - Candida Albicans, Coccidioides immitis, Cryptococcus neofromans - Histoplasma capsulatum - Pneumocystic jirovecii
	Parasitic	- Cysticercus cellulosae - Onchocerca volvulus - Toxoplasma gondii, Toxocara canis
Association with systemic non-Infectious diseases		- Behçet's disease, Blau syndrome - Crohn's disease - HLA B27-associated spondyloarthropathy - Juvenile idiopathic arthritis - Kawasaki's disease - Multiple sclerosis - Neonatal onset multisystem inflammatory disease - Psoriatic arthritis

TABLE 1. Continued.

Association with systemic non-Infectious diseases (Continued)	- Tubulointerstitial nephritis and uveitis - Ulcerative colitis - Vogt-Koyanagi-Harada syndrome - Reactive arthritis, Relapsing polychondritis - Sarcoidosis, Systemic lupus erythematosus
Ocular syndromes	- White-Dot syndromes* - Sympathetic ophthalmia - Pars planitis - Fuchs uveitis syndrome, Posner Schlossman syndrome** - Traumatic uveitis, Toxic uveitis***
Masquerade syndromes	- Neoplastic (lymphoma, retinoblastoma, leukemia) - Not neoplastic (retinal detachment, ischemia, pigmentary dispersion syndrome, retinitis pigmentosa, radiation retinopathy)

**Including Birdshot chorioretinopathy, multiple evanescent white dot syndrome, acute posterior multifocal placoid pigment epitheliopathy, multifocal choroiditis with panuveitis, serpiginous choroiditis, punctate inner choroidopathy and relentless placoid chorioretinitis.*

*** for the majority of these, the infectious agent has already been detected.*

**** Including topical prostaglandin analogues/ brimonidine, intravitreal triamcinolone actenoide/ anti-vascular endothelial growth factor, rifabutin, bisphosphonates, cidofovir, bacillus Calmette-Guerin vaccination, ipilimumab, pembrolizumab, nivolumab, anti-tumor necrosis factor agents (especially etanercept), fluoroquinolones.*

Grade of inflammation

Various classification systems have appeared for the severity of inflammation. In 1959, Hogan et al described for the first time a grading system for the inflammation of both anterior and posterior uveitis.[39,40] More classification systems for uveitis severity were added during this century and there was a clear need for a generally recognized and accepted system.[7,39-43]

In 2005, the Standardization of Uveitis Nomenclature (SUN) working group published a fundament for the now widely accepted classification system considering diverse aspects of uveitis, focusing mainly on anatomic location of uveitis. The classification of the duration and intensity of inflammation were developed. For the grading of the vitreous haze a classification was proposed by Nussenblatt et al which requires the comparison of the patient's features to the standard photographs. In clinical practice however the old system of Hogan and Kimura (4 grades in intensity) is commonly used for grading of vitreous inflammation.[39,41] The location of retinal lesions is currently being assessed using retinal zones according to a system developed by Holland et al.[44]

Anatomic classification of uveitis

The SUN working group agreed that the classification of anatomic location of uveitis should be based on the primary site of inflammation. The anterior portion of the uvea includes the iris and ciliary body, and the posterior portion of the uvea is known as the choroid (Figure 1).

Uveitis is commonly classified into anterior, intermediate, posterior or panuveitis (Figure 2).[45] The term panuveitis should be reserved for cases in which both, anterior and posterior segments are involved and there is no predominant site of inflammation. Inflammation in the anterior chamber and vitreous, but without involvement of chorioretinal lesions, should be referred to as anterior and intermediate uveitis, but not as panuveitis. This recommendation however was not followed and most ophthalmologists assign this type of uveitis either as intermediate or panuveitis, which might lead to confusion in this particular anatomic type.
The causes of uveitis are associated with the localization of the inflammation and in consequence identifying the primary site of inflammation may narrow the differential diagnosis (Table 2).

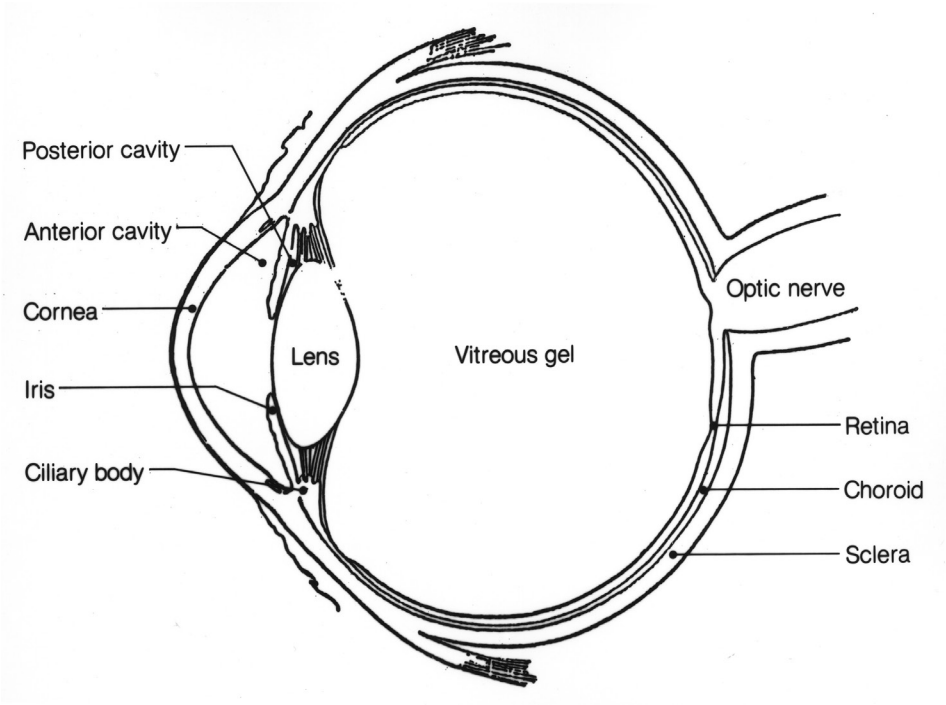

FIGURE 1. Anatomy of the eye.

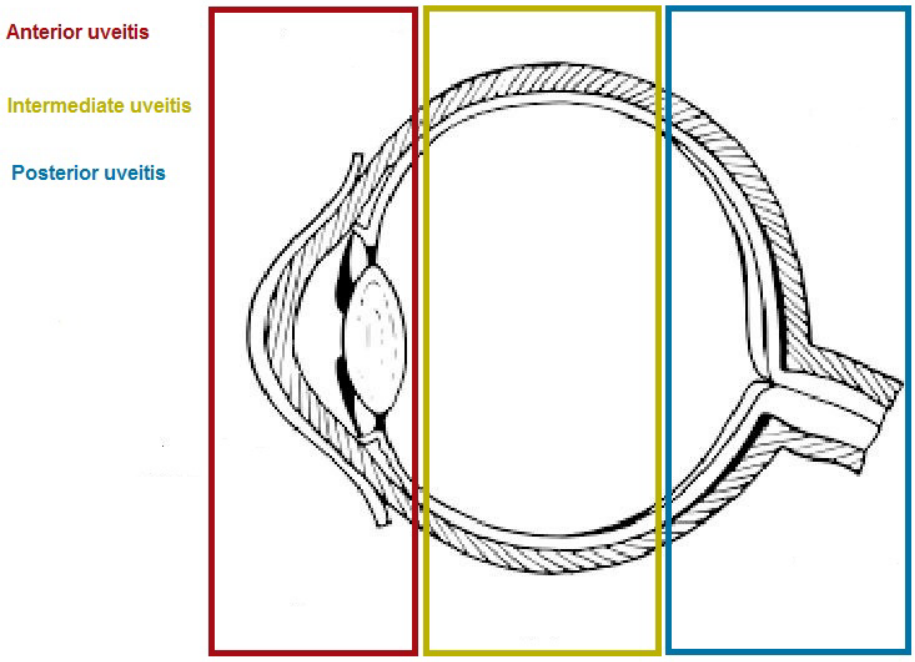

FIGURE 2. Anatomical classification of uveitis.

Rationale of the diagnostic work-up of uveitis patients

Determination of the cause of uveitis is challenging. In the past, multiple tests were performed in all patients with uveitis. However such an extensive work-up did not prove to be efficient. Many employed tests were non-contributory and did not help to find the underlying cause.[45-50] For example, toxoplasma serology was formerly included in the screening of uveitis patients, but did not prove to be efficient, as a large proportion of the Dutch population appeared seropositive and a positive serology was not discriminatory for ocular disease.[51]

Diagnostic tests should focus on the most common and treatable causes of uveitis.[45-50] The crucial first step is to make timely difference between an infectious and non-infectious cause and rule out masquerade syndromes.[52-54] This facilitates treatment decisions early in the disease course (e.g. immunosuppressive treatment in a patient with infectious uveitis may cause detrimental effects). Another aspect is to diagnose and treat an underlying systemic disorder.

Current diagnostic work-up

According to the Dutch national uveitis guideline, which was developed in 2015 and is being regularly updated, the diagnostic work-up should take place in all patients with uveitis of

unknown cause, except patients experiencing their first episode of mild anterior uveitis that reacts well to initial treatment.[55] The etiologic spectrum of uveitis implicates a multidisciplinary approach, but the treating ophthalmologist mostly initiates the initial diagnostic work-up.

TABLE 2. Differential diagnosis of uveitis according to its anatomical localization.

	Primary Sit of Inflammation	**Infectious Differential Diagnosis**	**Noninfectious Differential Diagnosis**
Anterior Uveitis	Iritis Iridocyclitis Anterior cyclitis	HSV VZV RV CMV *M.tuberculosis* Treponema pallidum	HLA B27-associated uveitis Reactive arthritis IBD JIA Behçet's disease TINU-syndrome Sarcoidosis
Intermediate uveitis	Pars planitis Posterior cyclitis Hyalitis	Borrelia burgdorferi	Multiple sclerosis IBD Sarcoidosis
Posterior uveitis	(multi)Focal or diffuse choroiditis Chorioretinitis Retinochoroiditis Retinitis Neuroretinitis	Toxoplasma gondii HSV VZV CMV Borrelia burgdorferi Bartonella henselae *M.tuberculosis* Treponema pallidum	Birdshot chorioretinopathy Multiple sclerosis Sarcoidosis Vogt-Koyanagi-Harada disease Behçet's disease TINU-syndrome IBD
Panuveitis	NA	Toxoplasma gondii VZV HSV Treponema pallidum *M.tuberculosis*	Sarcoidosis Behçet's disease Vogt-Koyanagi-Harada disease IBD Sympathetic ophthalmia

HSV = herpes simplex virus, VZV = varicella zoster virus, RV = Rubella Virus, CMV = cytomegalovirus, HLA = human leukocyte antigen, JIA = juvenile idiopathic arthritis, TINU = tubulointerstitial nefritis and uveitis, IBD = inflammatory bowel disease, NA=not applicable.

The recommended tests depend on the anatomical classification of uveitis as every anatomic location of uveitis yields a different differential diagnosis and in consequence a different initial work-up (Table 2 and 3).[45-50,56] Additional tests should be ordered based on ophthalmologic appearance of uveitis and the clinical history (tailored approach).

Indirect testing is common in uveitis, as direct evidence from the eye itself is hard to get. *Treponema pallidum* serology (TPHA/TPPA) may indicate syphilis and QuantiFERON-Gold

(QFT-G) or Mantoux testing an infection with *Mycobacterium tuberculosis*. Serum angiotensin converting enzyme (ACE) may indicate sarcoidosis and an additional chest X-ray may suggest sarcoidosis or TB. Nearly every type of uveitis has a potential association with sarcoidosis, syphilis or tuberculosis and diagnostic tests indicating these diseases should always be ordered in adult patients for any uveitis type. Several nonspecific blood tests are also included in the initial work-up of every uveitis patient, such as the complete blood count (in which leukocytosis may indicate systemic infection), liver and kidney function parameters (originally used in order to detect liver involvement in sarcoidosis patients), Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP)- both nonspecific parameters of inflammation. These parameters are also of interest before the initiation of therapy with systemic immunosuppressive agents, just like the detection of a (latent) tuberculosis infection by QFT-G testing.

HLA B27 is present in 50% of patients with acute anterior non-granulomatous uveitis, but can be also associated with scleritis or panuveitis. However, it should be kept in mind that 8-10% of Caucasians carry the HLA B27 antigen.[57] HLA B27 should be investigated in pediatric patients with uveitis, as its presence forms a strong risk factor for the development of enthesitis-related arthritis, psoriatic arthritis and an extended course of oligoarthritis.[57-59] HLA A29 is present in 5-7% of the general population and should therefore only be determined in patients with posterior uveitis and findings on funduscopic examination suggesting birdshot chorioretinopathy (BSCR), for which the presence of HLA A29 is typical.[60]

Anti-neutrophil cytoplasmic antibodies (ANCAs) should be determined in scleritis, and may indicate ANCA-associated small vessel vasculitis such as seen in granulomatosis with polyangiitis (GPA; which affects small and medium-size vessels). ANCAs are antibodies directed against intracellular proteins of neutrophil granulocytes and are subdivided into cytoplasmic-ANCAs (c-ANCAs) and perinuclear-ANCAs (p-ANCAs). More specifically, c-ANCAs are associated with GPA and p-ANCAs with diverse forms of vasculitis such as ulcerating colitis and retinal vasculitis.[61,62]

Rheumatoid arthritis (RA) is the most common systemic disease associated with scleritis. Rheumatoid factor (RF) and anti-cycli citrullinated peptides (CCP) may indicate RA and should be determined patients with scleritis.

Anti-nuclear antibodies (ANAs) are typical for JIA-associated uveitis in the pediatric population and should also be determined in scleritis. ANA is not distinctive for any specific uveitis causes in adults and might be also present in the normal population, especially in elderly females. However, it may have diagnostic value in patients with signs suggesting specific systemic diseases such as systemic lupus erythematosus (SLE).63 SLE causes typically retinal vascular disease and in fact not a genuine uveitis.

Advanced and costly tests or tests with potential side effects should be reserved for patients in whom these tests have therapeutic consequences. Any advanced imaging (Computerized Tomography (CT)- scan, Magnetic Resonance Imaging (MRI)-scan, Somatostatin Receptor Scintigraphy (SRS), etc.), Human Immunodeficiency Virus (HIV) serology are required only in second instance or earlier according to the patients history or clinical features.

TABLE 3. Initial diagnostic work-up for adult uveitis patients based on anatomic location.

	Anterior uveitis	Intermediate uveitis	Posterior uveitis	Panuveitis	Scleritis
ESR, CRP, general blood count, liver function, kidney function	+	+	+	+	+
HLA-B27	+	-	-	+	+
ACE	+	+	+	+	+
Treponema serology (TPHA/ TPPA)	+	+	+	+	+
QuantiFERON IGRA/ mantoux test	+	+	+	+	+
ANCA	-	-	+	-	+
Rheumatoid factor/ anti-CCP	-	-	-	-	+
ANA	+	-	-	-	+
Chest X-ray	+	+	+	+	+

ESR = erythrocyte sedimentation rate, CRP = c- reactive protein, HLA = human leukocyte antigen, ACE = angiotensin converting enzyme, IGRA = interferon gamma release assay, ANCA = anti-neutrophil cytoplasmic antibodies, CCP = cycli citrullinated peptides, ANA = anti-nuclear antibodies.

Intra-ocular fluid analysis

Diagnosis of infections

The diagnosis of infectious uveitis cannot be based on results of serology as the results from peripheral blood are not always indicative of the situation in the isolated eye.[51,64-66] Additionally, the value of the serologic tests depends on the prevalence of seropositivity in the population. This means that in a population with high seropositivity of an infectious agent, such as *Toxoplasma gondii* in the Netherlands, the value of positive serology in peripheral blood will be low. In these cases a diagnostic examination of intraocular fluids is worthwhile for identifying an infectious cause of uveitis. Especially in patients with threatened visual acuity and suspicion of an infectious cause, ruling out an infectious cause may accelerate the start of the correct therapy.

An anterior chamber tap might be useful even for the diagnosis of infectious posterior uveitis. In cases with strong suspicion of infection and negative anterior chamber tap a diagnostic

vitrectomy might finally reveal the underlying cause.[67]

Intraocular fluid is being analyzed by polymerase chain reaction (PCR) and/ or antibody detection. A PCR analysis is especially useful in patients with an underlying immunodeficiency disorder/ immunosuppressive treatment and for the detection of herpes viruses. Goldmann-Witmer Coefficient (GWC) seems especially useful in Rubella virus and *Toxoplasma gondii*. The GWC compares the relative percentage of specific antibodies in serum and eye. A positive GWC is indicative of active intraocular production of specific antibodies. Cultures are seldom useful in uveitis and are more of interest in suspicion of endophthalmitis.[53,68-70]

Diagnosis of lymphoma

Vitreoretinal lymphoma (VRL) is the most common malignancy masquerading as uveitis.[71] Diagnosis poses a challenge to ophthalmologists. Intraocular fluid might be used for cytologic examination or cell surface marker determination by flow cytometry. Cytologic findings indicative of VRL are large atypical lymphoid cells with large and irregular nuclei and multiple nucleoli.[72,73] Flow cytometry might be used to detect monoclonal lymphocyte populations using antibodies specific to B-lymphocyte markers (CD19, CD20, CD5, CD10 and κ/ λ light chains) as most primary intraocular lymphoma's are of B-cell origin.[73-75] Additionally, MYD88 mutations are frequent (60-80%) in VRL and may be detected by allele-specific PCR. In combination with CD20+ cells in the vitreous, a diagnosis of VRL can be confirmed.[76]

However, the usefulness of both cytologic examination and flow cytometry is limited to specimen gained by vitreous biopsy. Even then, cytologic analysis and flow cytometry can be difficult because of the sparse cellularity of vitreous specimens.[77]

A supplementary diagnostic method includes cytokine analysis for the determination of IL-10 and IL-6, which can also be determined in intraocular fluid gained by aqueous humor tap. The elevated ratio of IL-10/IL-6 raises suspicion of intraocular lymphoma.[75,78]

Current gaps in the knowledge of the diagnostic work-up

The Dutch national uveitis guideline advises on the diagnostic work-up of uveitis patients. However, some of the included diagnostic tests as well as their value in the work-up for new uveitis patients were so far not systematically studied.

With the rise of novel diagnostic tests in the past decades (i.e. diagnostic aqueous humor analysis, QFT-G testing), diverse infectious agents were implicated in uveitis, such as Rubella Virus, Epstein-Barr Virus and M. tuberculosis. The clinical spectrum of uveitis caused by Rubella virus is not known since the previous studies had a strong inclusion bias and included mostly patients with Fuchs Uveitis Syndrome. Despite the multiple case series, EBV-

associated uveitis and (latent) *M.tuberculosis* infection-associated uveitis remain an enigma, and the clinical characteristics of uveitis caused by these agents are not well documented.

The tests for sarcoidosis in patients with uveitis are commonly employed, but their diagnostic value during an early stage of uveitis was not systematically studied (e.g. ACE and chest X-ray). Also the diagnostic value of novel test for sarcoidosis (the soluble interleukine-2 receptor; sIL-2R) was not well investigated. General inflammation markers like lymphocyte count, ESR and CRP were since long used in the diagnostic work-up of uveitis patients, but their diagnostic value in the uveitis population is not known.

Aims, scope and outline of the thesis

The major objective of this thesis was to gain insight into the diagnostic value of the current examinations used in adult patients with recent onset of uveitis. The secondary aim was to report on clinical manifestations of patients who tested positive with these examinations. To achieve this, we started by providing an overview of the visual prognosis and morbidity of newly referred uveitis patients during their first year. Subsequently we provide an overview of the clinical characteristics and epidemiology of ocular sarcoidosis and the diagnostic value of implemented diagnostic tests (ACE and chest X-ray) but also explore novel diagnostic possibilities (sIL-2R and lymphopenia) for sarcoidosis-associated uveitis. We further investigate the utility of QFT-G testing in a Dutch uveitis population and the ocular and systemic features of patients with uveitis and positive QFT-G test. Thereafter, we summarize the typical clinical manifestations of common types of viral anterior uveitis and delineate their common clinical characteristics. More specifically we focus on the clinical characteristics of Rubella-virus and possible existence of Epstein-Barr virus-associated uveitis. Finally, we evaluate the diagnostic value of nonspecific inflammation markers (ESR and CRP) in patients with recent onset of uveitis. With these investigations, we attempt to improve diagnostic means for this debilitating ocular disorder.

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chapter 2

Visual Outcomes and Ocular Morbidity of Patients with Uveitis Referred to a Tertiary Center During First Year of Follow-up

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Eye (Lond). 2016 Mar;30(3):473-80.

Abstract

Purpose To describe the visual outcomes and morbidity of newly referred uveitis patients.

Methods Retrospective cohort study of 133 newly referred uveitis patients with active uveitis who required care in a tertiary center for at least one year. Main outcomes were Best Corrected Visual Acuity (BCVA) at referral and one year after referral, duration of visual impairment, systemic medications used as well as all complications and surgeries during the first year of follow-up. Generalized estimating equation models was used to assess prognosticators for poor BCVA.

Results The mean age at onset of uveitis was 43 years. The proportion of patients with at least one eye with BCVA ≤0.3 decreased from 35% at referral to 26% (P=0.45) at 1-year follow-up. The mean duration of visual impairment in the first year after referral was 4 months per affected eye. At one-year follow-up, bilateral visual impairment was observed in 4% but at least one ocular complication developed in 66% and 30% of patients required at least one intraocular surgery. Systemic immunosuppressive treatment was required in 35% of patients and the mean number of visits to ophthalmologist was 11 per year while 8% patients required hospital admission. Prognosticators for poor visual outcome included surgery undergone before referral (OR, 3; 95% CI, 1-11; P=0,047), visual impairment at referral (odds ratio [OR], 21; 95% CI, 8-54; P <0.001), and glaucoma before referral (OR, 7; 95% CI, 2-28; P=0,007).

Conclusions Patients with severe uveitis had a favorable BCVA 1 year after referral with only 4% of patients having bilateral visual impairment. This, in contrast to the prolonged duration of visual impairment during the first year of follow-up and the demanding care.

Introduction

The visual burden of patients suffering from uveitis is essentially unknown. There is a lack of systematic data assessing visual outcomes in large series of patients with uveitis and the data published so far are based on cohort studies commonly without standardized follow-up.[1,2]

The optimal best corrected visual acuities (BCVA) in the statistics addressing the visual impairment are commonly indicated in incidence and prevalence numbers and most reports are based on the prevalence of low vision or blindness at one time point, such as the presenting vision used by the World Health Organization or BCVA in the first year or after treatment used in clinical settings.[1,2,3] The course of disease in uveitis patients is extremely variable and visual performance changes according to the development of exacerbations and/ or chronic disease. Although the optimal BCVA may remain useful and can reach a good level after the inflammation subsides, the degree and impact of low vision during the active (sometimes prolonged disease episodes) remains essentially unknown. These periods of disease activity associated with (temporary) decreased vision together with multiple treatments and surgical interventions represent a real disease burden.

The aim of this study is to describe the visual prognosis and the associated risk factors of a poor visual prognosis in patients with active uveitis newly referred to a tertiary ophthalmology department and treated there for at least one year with respect to the degree, duration and causes of visual impairment during the first year after referral.

Methods

Study population

We conducted a retrospective cohort study at the department of Ophthalmology of the Erasmus Medical Center (Rotterdam, The Netherlands), which is a tertiary referral center. The local Medical Ethics Committee reviewed this study and concluded that approval was not required. All data were extracted out of medical records of patients and the research has followed the Tenets of the Declaration of Helsinki. The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines were used to ensure the reporting of this observational study.[4]

From January 2010 to January 2013, all newly referred uveitis patients were identified by a coding system of the referred patients. Out of this population we identified eligible participants according to the following inclusion criteria: 1. Presence of active uveitis referred for diagnostic investigations and/ or treatment; 2. Follow-up in our center for at least 12 months after referral. We excluded patients with inactive uveitis or patients referred for other eye conditions than primarily uveitis, non-medical referral reasons. Patients with visual loss due to other causes than uveitis (for example, amblyopia) were excluded in the final evaluation.

Assessment of determinants and outcomes

At the first visit, all patients underwent a comprehensive ocular examination including the notation of the activity of uveitis, BCVA, pupillary reactions, slit lamp examination, intraocular pressure (IOP) measurement and fundoscopy as well as notation of type and modality of treatment. Poor visual prognosis was defined as having visual impairment (moderate and severe) at 1-year follow-up. At follow-up visits, at least the current treatment and results of a routine ophthalmological examination were noted. Uveitis was considered active if anterior chamber cells ≥1+ or vitreous haze ≥1+. In posterior uveitis, active chorioretinal lesions were defined as lesions with indistinct borders associated with vitreous cellular reaction of leakage on fluorescein angiography or presence of active vasculitis on fundoscopy or angiography.

All patients underwent a standardized diagnostic investigation protocol according to the localization of the inflammation. This protocol included erythrocyte sedimentation rate, blood counts, serum angiotensin-converting enzyme levels, serology for syphilis and Lyme disease as well as interferon gamma release assay (IGRA) test (QuantiFERON–TB Gold In-Tube test) and in those with anterior and panuveitis Human Leukocyte Antigen B27 testing. Radiologic chest imaging was also performed. According to the clinical manifestations, additional examinations were performed (tailored approach). The accepted international criteria were used to diagnose Behçet's disease and ocular sarcoidosis.[6,7] In short, the diagnosis of

definitive ocular sarcoidosis was given to patients that had histologically confirmed diagnosis and presumed sarcoidosis was diagnosed in patients with chest imaging suggestive for the diagnosis of sarcoidosis and no other explanation for the uveitis, but without available histological proof. The diagnosis of ocular toxoplasmosis was always confirmed by intraocular fluid assessment.[8-11] Diagnosis of presumed ocular toxoplasmosis was based on typical clinical features of unilateral focal necrotizing retinitis sometimes associated with typical old pigmented scars. All other specific diagnoses were performed according to current diagnostic criteria. Definitive anatomical classification was performed (e.g. localization and laterality of uveitis) according to the Standardization of Uveitis Nomenclature (SUN) Working Group, by reviewing the whole follow-up period.

Diagnoses were grouped into infectious and non-infectious diseases and in established clinical ocular syndromes (e.g. pars planitis, birdshot chorioretinopathy). Patients with established ocular syndromes and identified cause or association with systemic disorder (e.g. multiple sclerosis with intermediate uveitis or documented rubella virus infection in Fuchs heterochromic uveitis syndrome) were classified according to the cause of their uveitis and not according to their ocular syndrome. Patients with a positive IGRA test in the presence of otherwise unexplained uveitis were classified as of unknown origin and further specified as latent tuberculosis-associated uveitis.

The following patient characteristics were extracted at the time of referral: gender, race, age at onset of uveitis, age at referral to our center, duration of interval from onset of uveitis to referral to our tertiary center as well as already established causes of uveitis and/ or associated systemic diseases, BCVA at referral and results of full ocular examination, ocular co-morbidities and all complications of uveitis present upon referral. The main cause of visual loss during the follow–up was attributed to the first complication, which caused the visual impairment. Also, type, frequencies and duration of treatment modalities, complications and surgical interventions were registered.

During the first year of follow-up we assessed the degree and duration of visual impairment and how often the patients visited our department (only uveitis-related visits were counted). Visual impairment was classified into the following categories: 1. No visual impairment (BCVA >0.3); 2. Moderate visual impairment (BCVA 0.16-0.33) and 3. Severe visual impairment (BCVA ≤0.1).[12] The duration of each category of BCVA was measured as follows: the BCVA at visit 1 was taken and assumed 129 constant until the next visit and the time between the two visits was the duration of the measured BCVA.

The following outcomes were measured at 1-year follow-up: BCVA, activity of uveitis and all other ophthalmological findings and the newly established causes of uveitis and/or

associated systemic disorders. If a patient had a planned ocular surgery within the first year after referral, but the surgery was actually performed at the end of the first year, the BCVA after that ocular surgery was taken. In our retrospective data, no reliable distinction could be made between ocular hypertension and glaucoma. Glaucoma was defined as an IOP of >24 mmHg measured at least at two subsequent visits, which was combined with glaucomatous opticopathy.[5] Epiretinal membrane (ERM) and cystoid macular edema (CME) were diagnosed when proven on optical coherence tomography (OCT).

Statistical analysis

Continuous data are presented as mean standard deviation, whereas categorical data are presented as proportions. The effect of the exposure variables on low BCVA was analyzed using multivariate logistic regression analyses in which all exposure variables were included and stepwise regression was utilized. Generalized estimating equation was applied to account for the correlation between both eyes of the same patient. Next, odds ratios with corresponding 95% confidence interval were calculated. All statistical analyses were performed using SPSS software (version 22.0). A P-value of <0.05 was considered statistically significant.

Patients with missing data on BCVA were excluded from the analysis. For all calculations with BCVA data, we converted decimal Snellen BCVA to the logarithm of the Minimum Angle of Resolution (logMAR). For easier understanding the logMAR results were converted back to decimal Snellen VA and only Snellen VA were reported.

Results

A total of 401 patients with uveitis were referred to our center in the specified time window. Among those, 133 patients (219 affected eyes) met the inclusion criteria and formed the final study population and 268 patients were excluded (Table 1). For the analysis of duration of visual impairment, we excluded one eye of a patient who underwent an enucleation (not related to uveitis).

Patient characteristics

The demographics and specific diagnoses are given in Table 1. The duration of interval from onset to referral was 2.5 years (±0.2 years). In 65% of the cases, the inflammation of uveitis was bilateral. The percentage of those with anterior uveitis was 26%. Our study included one patient positive for Human Immunodeficiency Virus.

TABLE 1. Demographics and baseline characteristics of newly referred patients with uveitis requiring tertiary care for at least one year.

Total no. of patients	133
Total no. of eyes	219
Age at onset of uveitis (yrs)	
Mean (+/-SD)	42.6 (±18.1)
Median	43
Range	5-83
Age at referral (yrs)	
Mean (+/-SD)	45.1 (±18.3)
Median	47
Range	7-85
Male-to-female ratio	1:2.4
Uni- to bilateral ratio	1:1.8
Race	**N (% of total)**
Caucasian	88/133 (66%)
Black	21/133 (16%)
Asian	12/133 (9%)
Mixed race	2/133 (2%)
Other races*	7/133 (5%)
Unknown	3/133 (2%)
Anatomical localization	**N (% of total)**
Anterior uveitis	35/133 (26%)
Intermediate uveitis	13/133 (10%)
Posterior uveitis	27/133 (20%)
Panuveitis	58/133 (44%)

TABLE 1. Continued.

Etiology	N (% of total)
Associated systemic disease	**60/133 (45%)**
Sarcoidosis**	27/60 (45%)
HLA-B27-associated uveitis***	11/60 (18%)
Multiple Sclerosis	5/60 (8%)
Other****	17/60 (28%)
Established ocular entity	**17/133 (13%)**
Birdshot chorioretinopathy	4/17 (24%)
Hypertensive anterior uveitis	4/17 (24%)
Other*****	9/17 (53%)
Infectious	**14/133 (11%)**
Toxoplasmosis	6/14 (43%)
HSV and VZV-associated uveitis	5/14 (36%)
Other infectious causes******	3/14 (21%)
Idiopathic	**42 /133 (32%)**
Latent tuberculosis-associated uveitis	7/42 (17%)

SD = Standard Deviation, HLA- B27 associated uveitis = human leukocyte antigen-B27 associated uveitis.

** Includes 6 with North- African decent and 1 Hispanic patient.*

*** Includes 19 definitive and 8 presumed sarcoidosis*

**** Including patients with and without spondyloarthropathy*

***** Includes juvenile idiopathic arthritis (N=3), Vogt- Koyanagi- Harada syndrome (N =3), Behçet's disease (N =3), inflammatory bowel disease (N =2), systemic lupus erythematodes (N =1), granulomatous polyangiitis (N =1), scleroderma-associated uveitis(N =1), periarteritis nodosa (N =1), masquerade syndrome (N =1) and systemic sclerosis (CREST syndrome; N =1)*

******Includes Fuchs hetereochromic uveitis (N =2), pars planitis (N =2), white dot syndrome (N =2), phacogenic uveitis (N =1), serpiginous uveitis (N =1) and presumed ocular histoplasmosis syndrome (N =1).*

******* Includes 2 patients with rubella virus-associated uveitis and 1 patient with cytomegalovirus-associated uveitis.*

Patient characteristics and changes during follow-up

The ocular patient characteristics at referral and 1 year after referral are depicted in Table 2 and 3.The proportion of patients with visual impairment in at least one eye decreased from 47/133 (35%) to 34/133 (26%; P=0.45; Table 2). In this visually impaired group, severe visual impairment decreased from 24/133 (18%) to 21/133 (16%) at one year of follow-up. At one-year follow-up, 4% had bilateral visual impairment and 22% had unilateral impairment (out of which 14% severe; 18 patients). Active uveitis at 1-year follow-up was still present in 32%. Systemic treatment at referral was given to 16% of patients, which increased to 35% ($p < 0.001$) after one year. Non-steroidal immunomodulatory drugs were most commonly used (25%) at 1-year follow-up (in patients who needed systemic treatment), while at referral systemic corticosteroids were mostly prescribed (8%).

TABLE 2. Characteristics of patients at referral and after follow-up in a tertiary center.

	Patients followed > 1 year in tertiary center At referral (N=133) N (%)	**Patients followed > 1 year in tertiary center At 1-year follow-up (N =133) N (%)**	**Excluded patients* At referral (N=268) N (%)**	**Excluded patients At the end of FU** (N=268) N (%)**
Visual impairment in at least one eye	**47/133 (35%)**	**34/133 (26%)**	**62/268 (23%)**	**16/268 (6%)**
Bilateral visual impairment	**9/133 (7%)**	**5/133 (4%)**	**10/268 (4%)**	**6/268 (2%)**
Severe visual impairment (BCVA ≤ 0.1)	4/133 (3%)	3/133 (2%)	3/268 (1%)	4/268 (1%)
Moderate visual impairment (BCVA ≤0.3)	5/133 (4%)	2/133 (2%)	7/268 (3%)	2/268 (0.7%)
Unilateral visual impairment	**38/133 (29%)**	**29/133 (22%)**	**52/268 (19%)**	**28/268 (10%)**
Severe visual impairment (BCVA ≤ 0.1)	20/133 (15%)	18/133 (14%)	36/268 (13%)	18/268 (7%)
Moderate visual impairment (BCVA ≤ 0.3)	18/133 (14%)	11/133 (8%)	16/268 (6%)	10/268 (4%)
Missing values	**0%**	**0%**	**11/268 (4%)**	**76/268 (28%)**
Etiologic diagnosis established	26/133 (20%)	98/133 (74%)	Not specified	Not specified
Systemic immunosuppressive treatment***	**21/133 (16%)**	**47/133 (35%)********	**37/268 (14%)**	**25/268 (9%)**
Corticosteroids*********	10/133 (8%)	4/133 (3%)	9/268 (3%)	5/268 (2%)
Non-steroidal immunosuppressive agent (with or without corticosteroids)	7/133 (5%)	33/133 (25%)	19/268 (7%)	13/268 (5%)
Biologicals with or without corticosteroids and/or immunosuppressive agents	4/133 (3%)	10/133 (8%)	9/268 (3%)	7/268 (3%)
Missing values	**0%**	**0%**	**51/268 (19%)**	**80/268 (30%)**

Abbreviation: BCVA = Best Corrected Visual Acuity.

** Reasons for exclusion were a short follow-up period (N=216), no active uveitis at first visit (N=28), non-medical reason for referral (N=9), missing data (N=14) and one patient was excluded because he refused treatment and a diagnostic work-up.*

*** Mean follow-up time 5.31 months.*

**** Systemic immunosuppressive treatment does not include local treatment regimens(periocular and intraocular injections of predominantly of corticosteroids) and/or acetazolamide for macular edema and/or antibiotic treatment used for various infectious disorders.*

*****There were additional 17 patients with short-term systemic treatment between these two points, however without systemic treatment at 1-year follow-up.*

****** Dosage in all cases more than 10 mg prednisolone (or equivalent) per day.*

TABLE 3. Ocular surgeries and complications developed during first year of follow-up in a tertiary center.

	Newly developed complications/ new surgeries during first year after referral (N=133) N (%)
Number of patients with at least one intraocular surgery	**40/133 (30%)**
Total number of new surgeries	**51 (100%)**
Cataract extraction only	18/51 (35%)
TPPV only	15/51 (29%)
TPPV combined with cataract extraction	12/51 (24%)
Glaucoma surgery	4/51 (8%)
Total cataract extractions with or without TPPV	30/51 (59%)
Other	2*/51 (4%)
Total number of new complications	**158 (100%)**
Cataract**	35/158 (22%)
CME	27/158 (17%)
Epiretinal membrane	24/158 (15%)
Posterior synechiae	17/158 (11%)
Retinal scars***	12/158 (8%)
Secondary glaucoma	10/158 (6%)
Corneal edema	9/158 (6%)
Miscellaneous****	24 /158 (15%)

TPPV = Trans Pars Plana Vitrectomy, CME = Cystoid Macula Edema

**Includes 1 enucleation and 1 iris biopsy*

*** Cataract causing decrease of visual acuity*

****Including any localization/ size*

***** Includes iris atrophy (N=7), vitreous/ retinal hemorrhage and/or neovascularization (N=6), retinal detachment and/or defect hole including any localization/ size (N =3), opticopathy (N=3), corneal scars (N=2), band keratopathy (N=1), fibrovascular tumor (N=1) and phtisis (N=1).*

The development of new complications during 1-year of follow-up was noted in 66% of patients (see Table 3); the most frequent new complication was cataract and CME. All, except for 2 patients, with new onset CME had non-anterior uveitis. The characteristics of ocular surgery performed during follow-up are illustrated in Table 3. Ocular surgery during the first year of follow-up was indicated in 18% of the affected eyes. Combined, 59% of the ocular surgeries involved a cataract extraction, which shows that cataract extraction was the most required surgery in affected eyes. The mean duration of visual impairment during the first year after referral was 4 months (range 0.25-12 months) per uveitis eye. Severe visual impairment was present in 41 of 219 (19%) affected eyes and the mean duration of visual loss was 6 months (2.7 months) per uveitis eye during the first year after referral. A total of 70 uveitis eyes had moderate visual impairment (70/219; 32%) during the first year after

referral and the mean duration of visual loss in this group was 4 months (3.1 months). During follow-up, the mean number of visits to ophthalmologist per patient was 11 (range 2-23) per year and 8% of patients required hospital admission for systemic treatment of their uveitis.

Causes of visual impairment

Table 4 shows the most common causes of visual impairment. The main causes of visual impairment were CME, retinal scars and glaucoma, for both severe and moderate visual impairment.

Risk factors for poor visual outcome

In the multivariate analysis, the poor visual outcome at 1-year follow-up was associated with visual impairment at referral (OR, 21; 95% CI, 8-54; P <0.001) and glaucoma before referral (OR, 7; 95% CI, 2-28; P=0.007) and we found a borderline association with surgery undergone before referral (OR, 3; 95% CI, 1-11; P=0.047), while non-anterior uveitis, age, race, gender, having systemic disease, use of systemic treatment and CME were not associated with the visual outcomes at 1 year.

TABLE 4. Main causes of visual impairment one year after referral.

	Total
Total number of uveitis eyes with BCVA ≤ 0.3	**39/ 219 (18%)**
CME	12/39 (31%)
Retinal scars	6/39 (15%)
Glaucoma	5/39 (13%)
Other	16/39 (41%)
Total number of uveitis eyes with VA ≤ 0.1	**25/ 39 (64%)**
CME	7/25 (28%)
Retinal scars	4/25 (16%)
Glaucoma	4/25 (16%)
Other*	10/25 (40%)
Total number of uveitis eyes with VA >0.1 and ≤ 0.3	**14/39 (36%)**
CME	5/14 (36%)
Other**	9/14 (64%)

BCVA = Best Corrected Visual Acuity, CME = Cystoid Macula Edema

** Includes opticopathy (N=2), phtisis (N=2), retinal detachment (N=2), active uveitis (N=2), neovascularization (N=1) and a combined cause of severe visual impairment because of glaucoma, CME and active uveitis (N=1).*

***Includes retinal scar (N=2), active uveitis (N=1), glaucoma (N=1), masquerade syndrome (N=1), opticopathy (N=1), retinal detachment (N=1), a combined cause of moderate visual impairment because of cataract, CME and pre- existent amblyopia (N=1) and a combined cause of moderate visual impairment because of a retinal scare and pre- existent myopia (N=1).*

Discussion

We report on satisfactory 1-year follow-up visual outcomes in patients with active and chronic uveitis who were newly referred to a tertiary center and who required tertiary care during the first year after follow-up. Despite the favorable visual outcomes, the prevalence of complications and the intensity of ophthalmological care were enormously high. While severe bilateral visual impairment occurred in only 2% of patients, the majority of patients suffered from visual impairment during their first 193 year after referral (51%), severe and multiple ocular complications needed frequent visits to an ophthalmologist and commonly required intraocular surgery. The above findings emphasize that the care for patients with chronic uveitis in tertiary centers is demanding and requires huge ophthalmological investments in the form of time and resource utilization.

Previous studies on visual prognosis of uveitis differ in terms of included population and time at which the VA is measured. These studies are mostly cross-sectional and include all patients ever seen in the tertiary centers (see Table 5) and consequently indicate VA in various stages of uveitis and have no standardized point of measurement. In addition, usually a total population with uveitis from a tertiary center was studied, including the cases with long-term follow-up (and frequently compromised VA) creating a bias for more severe patients (since tertiary centers will keep the patients with poor VA while patients with satisfactory outcomes will be referred back to their ophthalmologists). The percentage of the patients with anterior uveitis is being commonly used as an indicator of the severity of included uveitis population: while studies from peripheral ophthalmologic centers are characterized by a majority (approximately 80%) of patients with anterior uveitis, the reports from tertiary centers include mostly lower percentages (see Table 5), which is in accordance with our findings.[1,2,13]

Our study included patients with active uveitis who required treatment in a tertiary center and a high proportion of subjects was excluded (67%) because the follow up was less than one year. It is highly probable that the visual outcomes for the whole uveitis population will even be better than in our population of severe and chronic cases (Table 2).

In the previous studies, the proportion of patients with visual impairment (VA≤0.3) in at least one eye varied from 25 to 35%[1,2,13,14] which is in concordance with 26% in the present series. Our findings on satisfactory visual outcomes are in agreement with the recent study of Tomkins- Netzer *et al*.[1] In the study of Durrani *et al*[2], a much higher proportion of visual impaired patients (VA≤0.3) was found (reaching 70%) which is explained by the fact that the authors included the whole uveitis population and included all moments of visual loss during the follow-up period reaching from 1 months to 30 years.

TABLE 5. Previous Studies on the Visual Prognosis of Uveitis Patients.

	Rothova et al. 1996	Bodaghi et al. 2001	Durrani et al. 2004	Tomkins-Netzer et al. 2014	Present study
No. of patients	582	927	315	1076	133
Included patients	All patients seen in a tertiary center in 1993 and followed for >1 year	Cross-sectional study*	All patients seen in a tertiary center during 1998-2000	All patients seen in a tertiary center during 2010-2014	Newly referred patients followed for at least one year during 2010-2013
Bilateral VA ≤0.1	4%	3%	22%	2%	2%
Bilateral VA ≤0.3	6%	Not specified	13%	6%	2%
Unilateral VA ≤0.1	14%	10%	35%**	Not specified	14%
Unilateral VA ≤0.3	11%	Not specified		Not specified	8%
Most frequent cause of visual loss	CME	CME	CME	CME	CME

VA = Visual Acuity, CME = Cystoid Macula Edema

** Patients with idiopathic uveitis of more than 3 months duration, VA<0.2 at first presentation and requiring systemic anti-inflammatory drugs with a minimal follow-up of 2 years.*

*** These patients had unilateral visual loss, no further categorization into severe and moderate visual impairment was given by the authors.*

Bodaghi *et al*[13] included only patients with severe chronic uveitis who had a poor BCVA at presentation, giving also rise to selection bias. Our findings on visual outcomes are better than the results of the study performed in the Netherlands almost 20 years ago with the similar inclusion criteria (the number of patients with bilateral VA≤0.3 in the present series being 5/133 patients versus 57/582 patients in previous series, P=0.026).[14] In addition, the former study had a higher percentage of patients (42%) with anterior uveitis than 26% in our present series. The improvement in visual outcomes over time might be explained by the change and development of treatment approaches.

The duration of visual impairment due to uveitis was to our knowledge examined only in the study of Durrani *et al.*[2] The authors reported the duration of approximately 66% of the follow-up time, which is roughly consistent with our results (4 months per eye/year). The difference can be most likely explained by the different inclusion criteria and follow-up duration (in the present study being one year after referral to a tertiary center). The VA measured at one time point is not an accurate measure and our study indicates that the duration of visual impairment could be a more accurate measure in terms of the burden of these patients.

Similar to the findings of previous studies, the mean age at presentation in our series was 43 years.[1,2,13,14] Together with the duration of visual impairment of 4 months per eye-year in our series; this group is likely associated with a significant socio-economic burden. Little is known about the exact costs of uveitis patients. A previous study estimated the average monthly costs of treated patients 226 with non-infectious uveitis in 2009 ranging from US$ 1144 to US$ 2689, depending on the treatment regimens, which indicates that monthly healthcare costs are similar to those with diabetes mellitus and cancer patients.[15-17] Moreover, the costs associated with uveitis care measured only costs of medications and did not include the costs associated with hospital visits and intraocular surgeries.

The most common new complications in the present series were cataract, CME and ERM, which is slightly different from the previous studies in which glaucoma took the third place.[13,14] This may be related to the early detection of mild ERM by introduction of the OCT scanning technique or by different registration of complications. Glaucoma occurred in 6% of all new complications within the first year, which is similar to previous findings.[13]

Our study points out CME, retinal scars and glaucoma as major causes of visual impairment in uveitis, which is consistent with previously published reports.[1,2,13,14] One of the previous studies reported corneal opacities (mostly band keratopathy) as a cause of visual impairment, something we did not encounter in our present population.[14] The better treatment over time and our inclusion criteria might explain this discrepancy. The previous studies indicate that poor visual outcomes were associated with having non-anterior uveitis. In the present series, the visual prognosis at the first year after referral did not differ for patients with anterior and non-anterior uveitis. This might be explained by the fact that the present study included solely patients with severe anterior uveitis requiring a follow-up of more than one year in a tertiary center. Although CME was the most common cause of visual impairment, having new onset CME was not associated with poor visual outcome. CME was a common complication in our series (38% at referral and/ or during one year follow-up) and included also cases in which VA was not compromised. It is probable, that the early detection of CME by the routine use of the OCT- scanning technique and more vigorous therapy in the early stages explain the higher prevalence of mild new onset CME and a lower impact on VA in our series.

The favorable visual outcome probably reflects the intensive treatment of our patients. While in the past corticosteroids were the most common drugs used, our patients received predominantly non-steroidal immunomodulatory drugs. Still, 48% of the patients did not receive any systemic immunosuppressive treatment during the first year after referral. These patients received various local treatment modalities (including periocular and intraocular injections of predominantly of corticosteroids) and/or antibiotic treatment used for various infectious disorders and/or acetazolamide for macular edema. However, the

design of our study does not allow any comparisons on treatment modalities over time and the causes of better visual outcomes are not yet identified. The percentage of patients with intraocular surgeries in our series is similar to that of previous studies.[1,2,13,14] The most frequent procedure was cataract extraction, which is also consistent with the previous reports.[1,2,13,14]

VA is not the only indication for the outcome in all uveitis entities, particularly in conditions such as birdshot chorioretinopathy in which the central VA may remain uncompromised during long time. Retrospective study design prevents the systematic evaluation of visual fields in our patients. In addition, given that uveitis is a chronic condition, 1 year is not a long enough time period to follow visual outcomes and longer follow-up studies are needed. Another possible limitation of our study is the heterogeneity of diverse uveitis entities included. However, we did not aim to report on visual prognosis of specific uveitic entities, but report on an overall burden of uveitis treated in tertiary center. We attempted to select a more homogenous population of patients than 258 previous studies and did not include all patients who were followed in a tertiary center.

Our study includes newly referred patients to the tertiary center of patients with a namely Caucasian ancestry. Because of reference bias, our results cannot be used for the general population of uveitis patients outside a tertiary referral center. However, our study population is similar to previous studies, which were predominantly performed in tertiary centers. The biases inherent to retrospective study design such as misclassification, treatment bias and confounding also apply. Misclassification of the duration of visual impairment could be an issue due to the retrospective design, as patients visual acuities were more frequently measured when they had visual impairment. Thus, the duration of impairment is related to how precise the fluctuation in visual acuity is measured, resulting in a more precise measurement for more severe uveitis cases.

In conclusion, we present results from a cohort of newly referred patients with active uveitis to a tertiary center and illustrate that a majority of patients develops ocular complications and (temporary) decreased vision during the first year after referral, and show that a substantial part of patients requires systemic treatment and intraocular surgery. However, the visual results at the end of the first year were favorable with only 4% of patients having bilateral visual impairment. Our findings show that the tertiary care for patients with uveitis is complex, time-consuming and requires vigilant follow-up of patients by ophthalmologists taking care of this population.

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chapter
3

Ocular Sarcoidosis and its Diagnostic Tests

chapter 3.1

Ocular Involvement in Sarcoidosis

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Semin Respir Crit Care Med. 2017 Aug;38(4):514-522.

Abstract

Ocular involvement in sarcoidosis occurs in approximately 40% and the eye is the presenting organ in roughly 20%. The course of ocular disease does not necessarily parallel that of systemic disease. Uveitis is the most common presentation and shows mainly a chronic course; anterior uveitis is associated with better visual prognosis than posterior localization. Painful bilateral anterior granulomatous uveitis most commonly occurs in black patients at younger age, while painless posterior bilateral involvement with peripheral multifocal choroiditis is commonly seen in elderly white females. Patients with posterior uveitis develop often ocular complications and central nervous system involvement. Vitritis, segmental periphlebitis, choroidal granulomas and peripheral multifocal chorioretinitis are often seen clinical features. Optic nerve involvement is uncommon, but if present, results often in poor visual outcome. Lacrimal gland and conjunctival involvement are also common and present clinically as dry eyes or remain asymptomatic with good visual prognosis. Sarcoidosis-associated uveitis is mostly managed by local treatment with steroid drops or periocular and intraocular steroid injections or with novel intraocular corticosteroid implants. Patients with sight-threatening disease or optic nerve involvement need systemic therapy. Systemic therapy is based on a step-up regimen where corticosteroids are used in the initial phase of the disease and if long-term treatment is required, steroid sparing immunomodulatory drugs are implemented such as methotrexate or biological agents. Despite the mainly chronic course, need for long-term treatment and frequent ocular surgeries in the majority of patients, the visual outcome of sarcoidosis-associated uveitis is fairly good if therapy has started on time.

Sarcoidosis

Throughout history, sarcoidosis started as a dermatologic mystery in 1869. This mystery even had a place in 'The Adventure of the Blanched Soldier' in Sherlock Holmes series as a skin disease in 1930.[1] In the meantime, sarcoidosis was recognized as a multisystem granulomatous disorder, in which every organ could possibly be involved, including the eye. Uveitis is the most common manifestation of ocular sarcoidosis (OS) and is a major cause of visual loss in European patients with uveitis.[2-6]

Epidemiology of Ocular Sarcoidosis

The prevalence of ocular involvement in sarcoidosis is around 40% (25-50%).[3,7-15] According to A Case Control Etiologic Study of Sarcoidosis (ACCESS) including only biopsy-confirmed sarcoidosis patients from the US, the initial presentation of sarcoidosis was related to sex, race and age, but also geographical differences were reported.[14] The incidence of systemic sarcoidosis was reported to be high in Northern European countries but lower in Japan.[16] The opposite is true concerning the prevalence of ocular involvement in systemic sarcoidosis; the highest was reported in Japan (up to 79%) whilst a lower prevalence was found in Northern Europe (±28%).[8,9,17-22] In large uveitis series, the prevalence of sarcoidosis-associated uveitis is 3-18%, again with slightly lower percentages encountered in Northern Europe (up to 10%) compared to Japan (up to 18%).[23-28] Other Asian countries report lower prevalence of sarcoidosis-associated uveitis when compared to Japan, but Europe and US show similar prevalences. [27,29,30]

Systemic sarcoidosis affects both sexes equally.[31] However, diverse extra-pulmonary manifestations including the eyes were associated with female gender. [14,32,33] One study from China described an extraordinary high prevalence of sarcoidosis-associated uveitis in females (male-to-female ratio of 1: 6.5), which is much higher than found in other clinical studies.[26,34-36] Systemic sarcoidosis is more common in black compared to white patients and OS is also more frequent in black being 2-20 times more prevalent than in white race. [6,14,37-41] However, no significant difference in the prevalence of OS between black and white patients was found in one study which included only biopsy-proven sarcoidosis patients.[35]

Mean age at onset of sarcoidosis-associated uveitis is 44-52 years and usually 2 peaks in age are reported, the first in the second or third decade of life and the second peak in the sixth decade. [35,36] However, females were older compared to male patients at onset of sarcoidosis-associated uveitis. [35,42,43] No difference in prevalence of OS was found under and above the age of 40 years in ACCESS, but in this study 'eye involvement' was broadly defined and included also extraocular disorders such as lacrimal gland enlargement and keratoconjunctivitis sicca.[14]

Specifically, the presentation of sarcoidosis-associated uveitis shows epidemiological differences. Black patients tend to be younger females (second and third decades) typically presenting with bilateral granulomatous anterior uveitis while white elderly females present most commonly with posterior segment involvement (showing peripheral multifocal chorioretinitis and cystoid macular edema). [11,14,15,17,35,43-52] However, white patients show also a peak in the second and third decades, with anterior uveitis being most common. [6,8,34,53-55] Asian patients seem to be older at onset (sixth decade) with predominantly posterior segment involvement and do not exhibit gender differences in ocular sarcoidosis. [36,56]

Diagnosis of Ocular Sarcoidosis

The presence of systemic sarcoidosis in a patient with unexplained uveitis is generally accepted as a confirmation of sarcoidosis-associated uveitis. The diagnosis of ocular sarcoidosis without systemic manifestations remains difficult as intraocular tissues are not easily available for biopsy (with the exception of extraocular tissues like the conjunctiva or lacrimal gland) and there is no single clinical feature exclusive for ocular sarcoidosis. The initiative to define specific criteria for the diagnosis of intraocular sarcoidosis (International Workshop on Ocular Sarcoidosis; IWOS) points out that some clinical signs are being suggestive for sarcoidosis (granulomatous uveitis, segmental periphlebitis). However, the IWOS diagnostic criteria remain to be validated in practice, and in fact sarcoidosis should be considered in all forms of uveitis.[57] Interestingly, one study shows that patients with sarcoidosis-associated uveitis had elevated intraocular angiotensin-converting enzyme levels while having normal serum values, but the value of intraocular fluid assessment in diagnosis of ocular sarcoidosis was so far not systematically investigated.[58]

Differential diagnosis of sarcoidosis-associated uveitis

Ocular disorders to be considered include principally tuberculosis, syphilis and viral infections. Tuberculosis may also be accompanied by granulomatous uveitis, multifocal choroiditis and intraocular granulomas. Systemic signs, imaging and laboratory examinations are helpful in distinguishing both entities. Birdshot retinochoroidopathy and Vogt-Koyanagi-Harada disease may show multiple retinal lesions resembling those in sarcoidosis. However, patients with birdshot retinochoroidopathy are typically Human Leukocyte Antigen-A29 positive and have no associated systemic signs. Vogt-Koyanagi-Harada disease shows associated features of encephalitis, inner ear involvement and has subsequent dermatologic manifestations (next to additional typical ocular signs such as serous retinal detachments).[59]

Clinical Presentation of Ocular Sarcoidosis

Ocular presentation of sarcoidosis

The eye is the presenting organ in sarcoidosis in approximately 20% (5-40%) of cases and uveitis is the most common ocular manifestation of sarcoidosis, followed by conjunctival and lacrimal gland involvement.[3,6,8,11,16,17,34,35,45,47]

Intraocular manifestations

Intraocular manifestations of sarcoidosis include (commonly bilateral) uveitis, which can be further classified as anterior, intermediate, posterior or panuveitis depending on the location of inflammation within the eye. Sarcoidosis-associated uveitis commonly has a smoldering chronic disease course with a minority showing monophasic disease or recurrent flares (2-7%).[34,43,60]

In general, patients with acute anterior uveitis present with visual loss, redness and pain.[61] Acute anterior uveitis in sarcoidosis occurs frequently in young patients and typical is its manifestation in Lofgren's syndrome. In contrast, chronic anterior uveitis or posterior segment involvement may lack all of these symptoms. In the past, anterior uveitis in sarcoidosis was typically classified as granulomatous (i.e. exhibiting large keratic precipitates on the cornea and granulomatous lesions on the iris or in the iridocorneal angle; Figure 1).

Koeppe nodules on the pupillary border, composed out of plasma cells, can also be seen (Figure 2).[43,62-65] Granulomatous anterior uveitis suggests the presence of sarcoidosis, but iris nodules can also be seen in other granulomatous disorders such as tuberculosis.[43,44,66] Posterior synechiae (adhesions between iris and lens; Figure 3) are frequent, but hypopyon is not typical for sarcoidosis-associated anterior uveitis.[67]

Intermediate uveitis is characterized by the inflammation of the vitreous body and is predominantly encountered in young adult patients.[27] Intermediate uveitis is often painless but patients complain about floaters and decreased vision and on examination demonstrate vitreous opacities, which are being described as snowballs and snow banking (accumulation of leukocytes and vitreous debris in the vitreous or on the peripheral surface of the retina).[57,62] These are however general signs of intermediate uveitis and differentiation from other types of intermediate uveitis in the absence of systemic signs typical for sarcoidosis might be difficult.

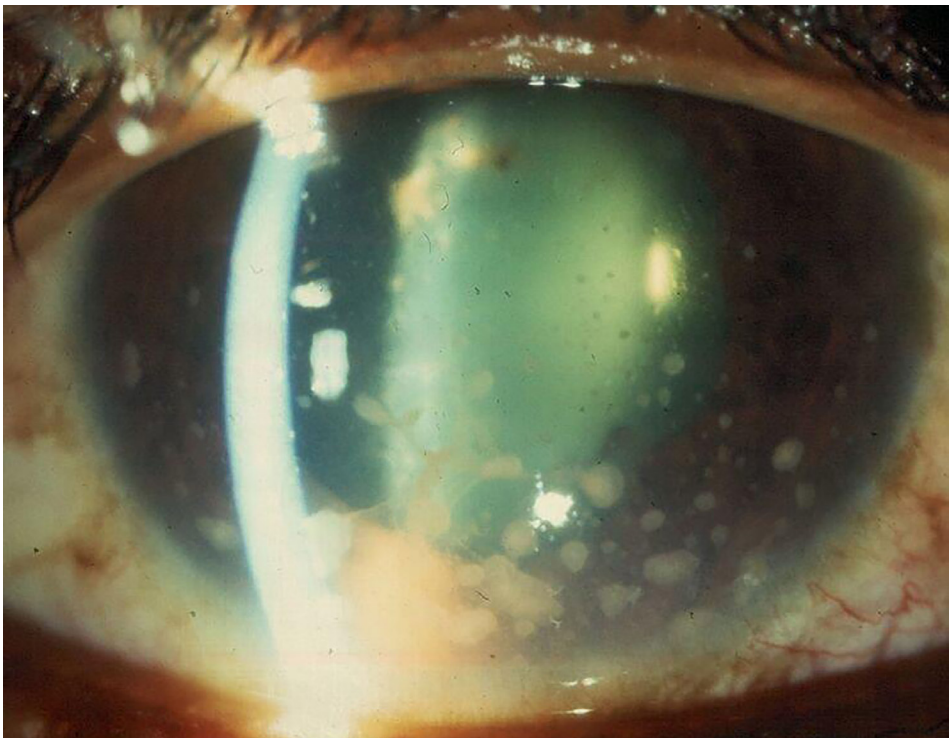

FIGURE 1. Granulomatous anterior uveitis in sarcoidosis with “muttonfat” keratic recipitates located on the corneal endothelium.

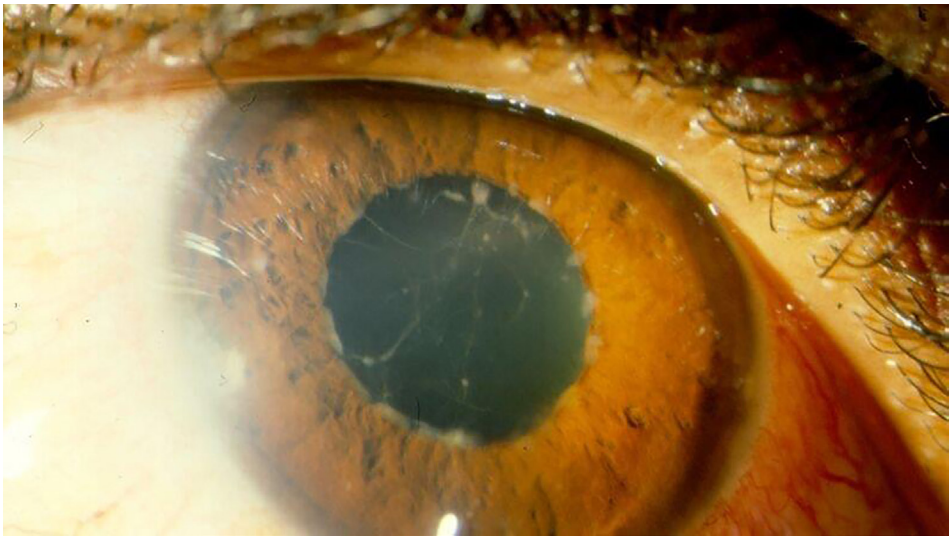

FIGURE 2. Granulomatous anterior uveitis in sarcoidosis exhibiting small nodules on pupillary border (Koeppe’s nodules) and fibrin strands in the pupillary aperture connecting these nodules. In general, the nodules are transient in character.

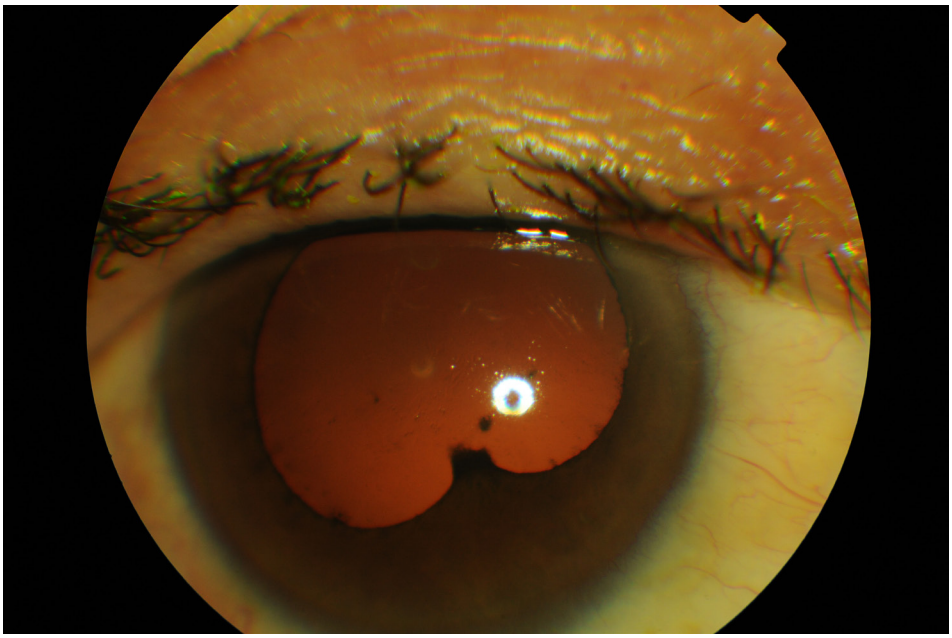

FIGURE 3. Posterior synechiae in a patient with uveitis (adhesions between the iris and the lens).

Posterior uveitis presents with painless visual loss and floaters. On examination, retinal phlebitis, multiple chorioretinal peripheral lesions and choroidal nodules (granulomas) can be seen.[57] Sarcoidosis-associated retinal phlebitis is commonly non-obstructive, with segmental vascular exudates around the veins (fundoscopically seen as candle wax drippings or vascular sheathing), usually localized in the midperipheral retina and its presence and activity can be confirmed by fluorescence angiography (Figure 4).[68-70] The clinical entity of occlusive retinal vasculitis associated with vitreous bleedings presents commonly in young male patients and can be associated either with sarcoidosis or with tuberculosis.[71] The clinical entity of peripheral multifocal chorioretinitis shows multiple punched-out lesions in the peripheral retina and occurs predominantly in elderly female patients (Figure 5).[72-74] Visual loss in these patients is common and is often caused by CME.[6,34,35,75,76] Granulomas can be located anywhere in the fundus and remain often asymptomatic if the optic nerve or macula are not involved (Figure 6). Hypopigmentation or scarring remains when the granuloma diminishes. However, the identification of asymptomatic granulomas may be of importance since the incidence of central nervous system (CNS) involvement is increased in patients with posterior uveitis.[50,60,77,78]

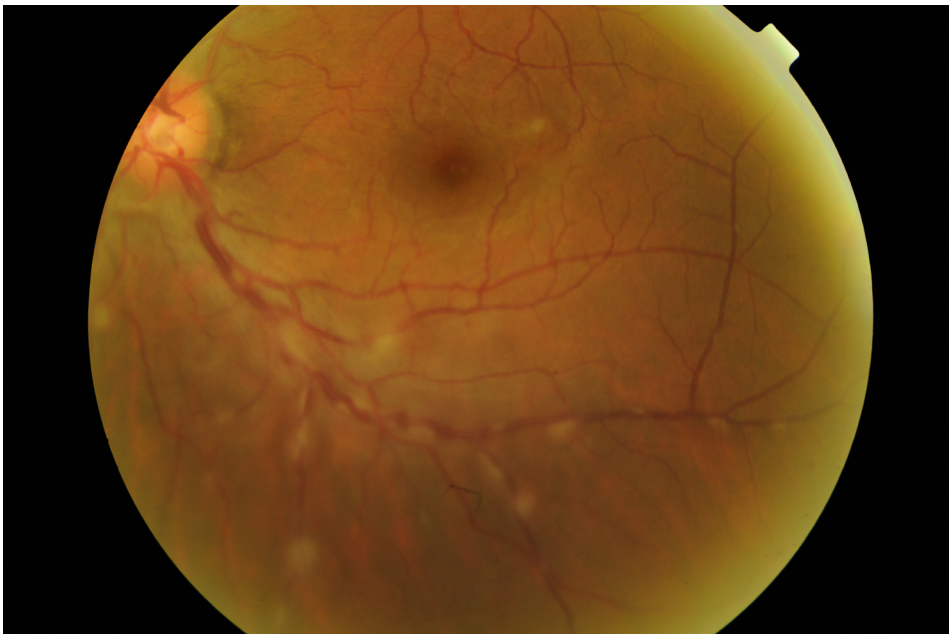

FIGURE 4. Segmental retinal vasculitis in a 39-year-old male patient presenting with peripheral facial nerve paresis and bilateral posterior uveitis, who was diagnosed with sarcoidosis. Note the segmental white fluffy lesions around the vessels, called "vascular sheathing." The patient reacted well to methotrexate in combination with prednisone.

Optic nerve involvement

In general, optic nerve involvement is uncommon (1-5%), but in neurosarcoidosis, cranial nerve involvement is the most common presenting feature (55%), with the optic nerve being most commonly affected (33-75%).[79-88] Post-mortem studies indicate even a higher prevalence of optic nerve involvement, which suggests higher rates of CNS involvement than clinically evident.[89] Solitary optic nerve involvement can present as acute or chronic, mostly painful visual loss.[79,90] Optic disc edema or optic disc pallor can be seen on fundoscopy.[83] The optic nerve can be directly infiltrated by inflammatory cells or its involvement may be secondary due to an adjacent granuloma compressing the nerve, or by meningeal inflammation (with papilledema, optic neuritis and elevated intra-cranial pressure; Figure 7).[91] All these conditions can result in optic nerve atrophy causing irreversible visual loss.[81] Even patients who receive timely high doses of corticosteroids may retain impaired vision.[91,92] In patients presenting with solitary optic nerve involvement, the disease may be confused with optic nerve meningioma.[86] However, concomitant inflammatory ocular and pulmonary involvement can help with differential diagnosis.[79,81]

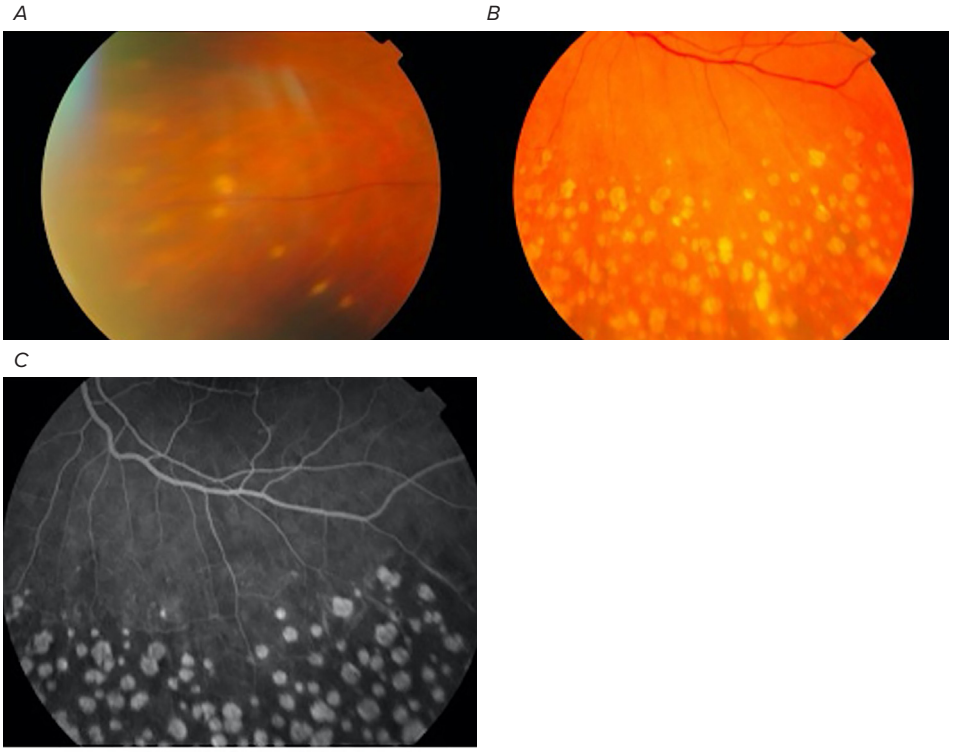

FIGURE 5. Peripheral multifocal chorioretinitis. (A) Chorioretinal lesions in a 67-year-old male patient located in the peripheral retina. The white small chorioretinal lesions have blurry borders. The hazy details are also caused by vitritis. (B and C) Peripheral punched-out lesions in a 70-year-old woman with sarcoidosis-associated posterior uveitis. (B) Multiple quiescent punched-out lesions after the resolution of chorioretinal infiltrates. Typically, these are round or oval in shape, sharply demarcated and located on the (mid-)peripheral retina, mostly inferior. (C) Punched-out lesions as seen on fluorescence angiogram.

Tattoo-associated uveitis

Tattoo-associated uveitis is associated with the development of non-caseating granulomas in skin tattoos. Most of the reported patients had no evidence of pulmonary sarcoidosis on chest X-ray. Whether this entity represents a sarcoid reaction to foreign antigens or a genuine sarcoidosis is a matter of debate; in 3 reported cases until now, ocular inflammation diminished after tattoo excision.[93-95] Asking for body tattoos, therefore, became a part of history taking in a patient with uveitis.

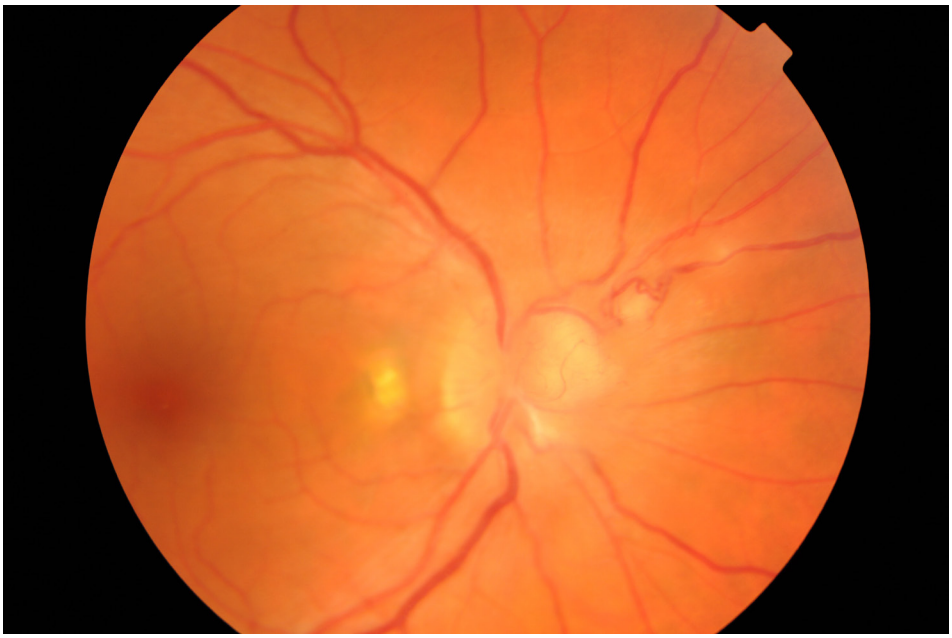

FIGURE 6. Granuloma located on and nasally from the optic nerve in a 42-year-old female patient with sarcoidosis-associated panuveitis in both eyes. The granulomas regressed under therapy with methotrexate.

Extraocular manifestations

The lacrimal gland and conjunctiva are the most commonly involved periocular tissues.[35,96] Histopathological studies of biopsy-proven orbital sarcoidosis show lacrimal gland involvement in 42-63%.[10,97,98] The lacrimal gland inflammation and subsequent atrophy results in decreased tear production and presents clinically as keratoconjunctivitis sicca (dry eye), but usually remains asymptomatic for a long time.[52,99-101] Typically, clinical evidence of local swelling is seen at the time of active inflammation.[3,47,102] Also the conjunctival involvement can cause dry eye or patients may present with conjunctival nodules, which react well to topical steroid treatment.[97,103] The prevalence of conjunctival involvement varies widely in the literature and this discrepancy may be in part attributed to variable definitions of conjunctival sarcoidosis and depends on how detailed the pathological examination of conjunctival tissue was performed.[35,96,97,103] Lacrimal gland and conjunctival involvement present mostly solitary without associated uveitis and may be used for biopsy confirmation due to their easy access.[3,35,47,102] The diagnostic value of undirected conjunctival biopsy (i.e. in cases without visible conjunctiva manifestations) remains a matter of debate.[36,104]

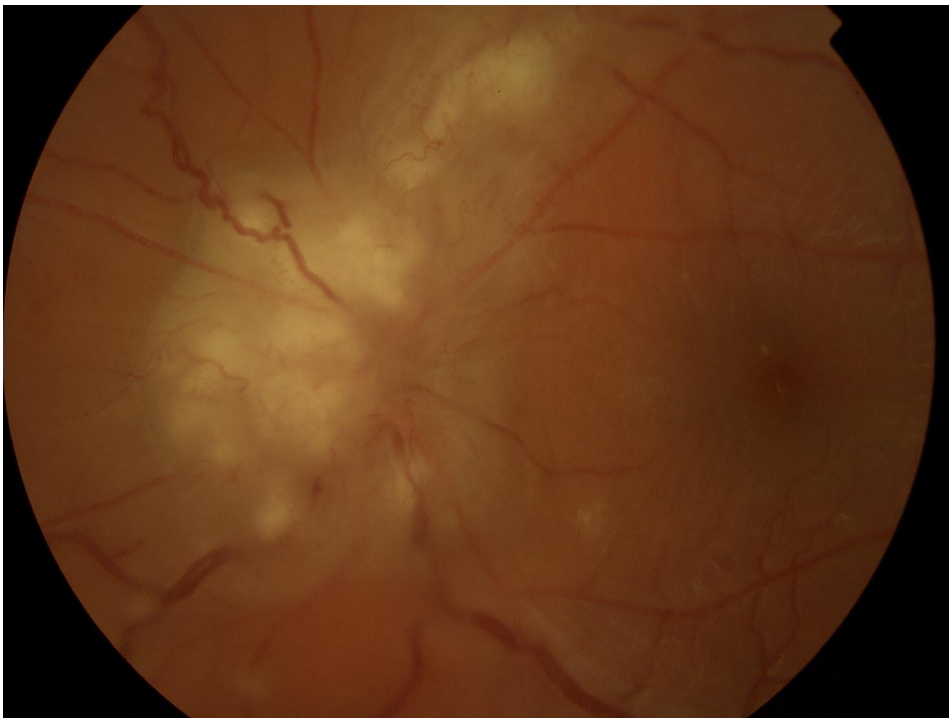

FIGURE 7. Optic nerve involvement in ocular sarcoidosis in a 31-year-old female patient. Note the peripapillary swelling, congested vessels,and associated subretinal whitish infiltrates.

Relation between ocular and systemic sarcoidosis

Ocular and systemic disease may have different time courses. The majority of patients with sarcoidosis-associated uveitis have already systemic evidence of sarcoidosis at the onset of uveitis (47%; mostly the lungs: 69%) and patients presenting with systemic sarcoidosis show ocular involvement at some time point during the disease in 4-50%.[16,105,106] However, uveitis can precede the non-ocular detectable signs of sarcoidosis in 31% by more than one year and systemic manifestations may develop later (during the first 5 years in 7-15% of patients, the most common being the skin and central nervous system).[6,105,107]

In patients with chronic systemic sarcoidosis, uveitis does not pursue a chronic course in 45%.[3] The severity of systemic involvement is not associated with the development of ocular sarcoidosis.[11,13,35,108] Only one study, to our knowledge, reports on a higher incidence of advanced stages of pulmonary sarcoidosis in patients with uveitis.[109] So far, there are no specific extra-ocular manifestations of sarcoidosis identified that show relationship with ocular involvement.[6]

Complications of Ocular Sarcoidosis

Cataract, glaucoma and macular edema are the most common complications in sarcoidosis-associated uveitis.[43,63,107] In addition, cataract and glaucoma represent the major ocular side effects of corticosteroids, which are commonly used. Whilst cataract causes temporary visual loss, which improves after surgery, macular edema and glaucoma -if not adequately treated- can cause permanent visual disability.[43]

Macular edema is the most common complication of OS (45-58%), mostly occurring in patients with posterior uveitis.[43,107] The prevalence of macular edema is correlated to the duration of active inflammation and delay of treatment.[43] Cataract was observed in approximately 24% of sarcoidosis patients at some time point during their disease course.[43,63,105] Secondary glaucoma develops in 20% of patients with sarcoidosis-associated uveitis and about 30% of these require surgical treatment.[43,63,105] Retinal neovascularization is usually reported as an infrequent complication (4% in posterior segment involvement with ischemic vasculitis), is mostly located on the optic disc and requires usually laser therapy.[43,63]

Therapy

The principal treatment approach in OS is to start with local treatment comprising corticosteroid drops and/or injections or implants and subsequent switch to systemic therapy in patients with insufficient response to local treatment modalities. Successful management of sarcoidosis-associated uveitis with local treatment was achieved in approximately 50% of cases.[43,63,105,107] Primary systemic therapy is indicated in patients with sight threatening disease and in those with optic nerve involvement. In addition, systemic therapy is used for severe chronic ocular disease, patients resistant to local treatment regimens and/ or showing intolerance for local treatment modalities.[110,111] Approximately half of OS patients need systemic treatment for ocular inflammation alone and an additional part needs systemic therapy for ocular inflammation and active systemic disease.[107]

Local treatment

Corticosteroid eye drops are used together with cycloplegic eye drops (for prevention of synechiae and against ciliary spasm, which causes pain) in anterior uveitis. Common side effects of topical steroids include early cataract formation and raised intraocular pressure.[62]

Intraocular corticosteroid injections and implants

Periocular and intraocular corticosteroid injections or implants may be given to patients with involvement of the posterior eye segment. Periocular triamcinolone injections are most commonly used, which have approximately an effect duration of 3 months.[112] Dexamethasone intraocular implant (Figure 8) is an alternative option for treatment of inflammation as well as CME. Following the intravitreal injections, patients are monitored for elevation of intraocular pressure [113-115] Fluocinolone acetonide implant has longer effect duration, but is more expensive and has to be placed surgically.[116] The side effects of intraocular implants include frequent development of cataract and glaucoma, which require ensuing surgeries. Endophthalmitis occurring after intraocular injection is exceptional, but can be devastating for visual outcome.

Systemic treatment

The mainstay of the systemic therapy in OS consisted so far of corticosteroids. In the past, only a minority received an additional immunosuppressive therapy, but at present, corticosteroid-sparing treatment regimens are recommended for all patients with intraocular inflammation who require systemic treatment for a longer period than 3 months.[43,63,105,107,117,118] Use of systemic steroids is inversely associated with poor visual outcomes indicating that early treatment is important.[43] Intravenous methylprednisolone may be used in patients with imminent visual loss.[62] However, the side effects (ocular and systemic) of corticosteroids are common and limit their long-term use.

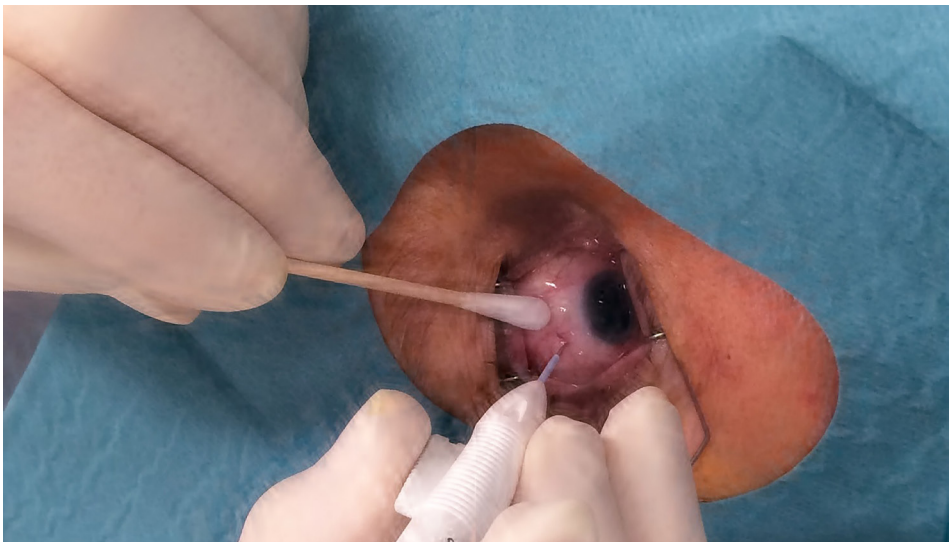

FIGURE 8. Injection of intravitreal dexamethasone implant under topical anesthesia with eye drops.

Various systemic immunomodulatory treatment options were recommended for OS including methotrexate (MTX), azathioprine, mycophenolate mophetil (MMF) as well as anti-tumor necrosis Factor (TNF) alpha agents. Because most of these agents need several weeks to reach their full effectiveness, initial combination with temporary administration of corticosteroids is recommended.[117] MTX has become a widely used steroid-sparing agent with reported treatment responses ranging between 39-100%.[119-123] Azathioprine and MMF were scarcely investigated in OS. All of the above mentioned drugs demonstrated an improvement of visual acuity at some point during the follow-up.[124-126] One retrospective cohort (including 257 patients but without specifying the proportion of sarcoidosis-associated uveitis patients) comparing MTX, azathioprine and MMF for non-infectious uveitis showed MMF to have a higher and more rapid response rate than MTX, however studies on MMF efficacy in OS are lacking.[127]

Anti-TNF agents were shown to be effective in OS. However, most evidence on efficacy of anti-TNF agents comes from studies with nonspecific uveitis; only occasional studies assessed their efficacy in sarcoidosis-associated uveitis. Most commonly used biological drugs in OS were infliximab and adalimumab.[128-131] Etanercept is not recommended for uveitis and was even reported to induce uveitis in sporadic cases.[132-137] Adalimumab has been found effective in sporadic patients with Blau syndrome.[138] In contrast, adalimumab-induced sarcoidosis (including ocular manifestations) has also been reported in patients treated with adalimumab for other immune-mediated disorders.[139] The most recent and largest observational study in uveitis (however including only 10/160; 6% sarcoidosis patients), reported adalimumab and infliximab to have similar treatment results.[140]

Prognosis

The visual prognosis of sarcoidosis-associated uveitis is fairly good. The median visual acuity at presentation has been reported to be 0.4, which increased to 0.6 at a later stage.[141] In reports, which determined visual acuity at two time points, visual acuity of less than 0.5 (for driving license required >0.5) was noted at onset in approximately 50% of patients and improved to 30% after treatment.[43,63,142] The most common causes for visual decrease consisted of CME and cataract.[3,6,43,47,60,63,107,141,143] Important risk factors for poor visual outcome are posterior localization of uveitis and black race.[3,6,43,60] Complications associated with poor visual outcomes include CME, optic neuropathy, glaucoma and development of neovascularization's.[3,43,48,63,141] Most importantly, lack of timely treatment is strongly associated with lack of improvement in visual acuity.[43]

Interestingly, the 1-year all-cause mortality was lower in sarcoidosis patients with ocular involvement compared to those without ocular inflammation.[45]

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chapter

3.2

Chest Radiographic Screening for Sarcoidosis in the Diagnosis of Patients with Active Uveitis

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Ann Am Thorac Soc. 2017 Jun;14(6):912-918.

Abstract

Rationale Although chest radiography is currently recommended for the initial evaluation of patients with new onset uveitis, the efficacy of this diagnostic screening modality is not known.

Objective To evaluate the diagnostic value of chest radiographs in patients with active uveitis of recent onset in a tertiary center in Western Europe.

Methods A retrospective cross-sectional study was conducted by reviewing all chest imaging for adults with new onset (<1 year) uveitis of unknown origin undergoing initial evaluation in the Department of Ophthalmology at Erasmus University Medical Center (Rotterdam, the Netherlands). Radiographic findings were related to clinical and other imaging characteristics and to final diagnoses.

Results Screening chest radiographs were abnormal for 30 of 200 (15%) patients included in this study. Twenty-two of the 200 patients (11%) had biopsy-confirmed sarcoidosis and an additional 14 patients were presumed to have sarcoidosis. The finding of chest radiographic abnormalities interpreted as typical for sarcoidosis was specific (91%; 95% CI; 85.9-94.4%) but not sensitive (64%; 95% CI; 43.0-80.3%) for biopsy-confirmed sarcoidosis. The combination of an elevated serum angiotensin-converting-enzyme level and chest radiographic findings typical for sarcoidosis increased the sensitivity to 79%. Biopsy-confirmed sarcoidosis was more common in patients with panuveitis (17/84; 20%) compared to patients with other anatomical locations of uveitis (5/116; 4%, P<0.001). One patient was diagnosed with active pulmonary and ocular tuberculosis.

Conclusions Abnormal chest radiographs were found in 15% of patients with active uveitis of unknown origin and onset within one year of referral to a tertiary center in the Netherlands. A majority of the abnormal chest radiographs showed findings compatible with the diagnosis of sarcoidosis.

Introduction

Determining the cause of acute uveitis is critical for management and prognosis. However, which specific diagnostic tests should be performed during initial diagnostic evaluation is not clear. Based on limited data, a "tailored screening approach" is widely recommended for patients with uveitis. [1-4] This approach includes specified screening studies depending on the intraocular location of inflammation and is further determined by specific characteristics of individual patients as revealed by a medical history and a multi-system physical examination.

Chest radiographs are commonly obtained during the initial evaluation of patients with the exception of patients with a first attack of mild-anterior uveitis, However, there is no published evidence supporting the efficacy of this strategy.[5-7] In this study, we report on the utility of chest radiographic screening for 200 patients who were referred to a tertiary medical center for diagnostic assessment and treatment of active, recent-onset uveitis of undetermined cause.

Methods

This cross-sectional study was performed by members of the Department of Ophthalmology at the Erasmus University Medical Center in Rotterdam, the Netherlands. Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines were used to structure the reporting of this observational study.[8] The research followed the tenets of the Declaration of Helsinki and was approved by our medical ethics committee.

Participants

We reviewed the medical records of patients referred for evaluation and treatment of uveitis to our tertiary medical center between 2012 and 2015 (N=1210). We included patients with active uveitis of recent onset (<1 year) as the primary presenting problem. The inclusion of patients is depicted in Figure 1. Patients with a known etiologic diagnosis for uveitis at the time of referral and patients with a uveitis duration of more than one year were excluded.

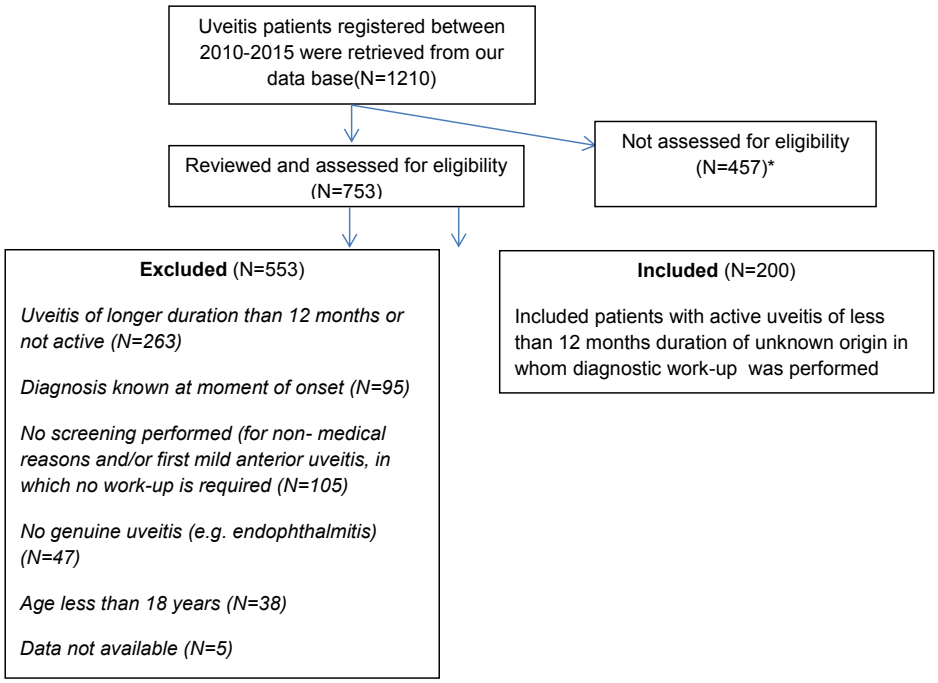
We stopped accruing patients when the number of study subjects reached 200 because the proportions of radiologic sub diagnoses did not change as accrual approached that number. Our study comprised only patients with uveitis of unknown origin, and only the results of the first diagnostic evaluation for the cause of uveitis were included. Posteroanterior and lateral chest radiographs were performed on all patients during the initial diagnostic evaluation (Figure 1) before any systemic treatment was initiated. Chest computed tomographic (CT) imaging and somatostatin receptor scintigraphy were performed if the initial diagnostic evaluation raised a suspicion of sarcoidosis.

Clinical and laboratory assessment

The onset of disease was defined as the date when an ophthalmologist first ocumented uveitis. We recorded the age and sex of the patients, the location of uveitis within the eye, laterality, and the duration of uveitis before referral to our center. Definitive anatomical classification was determined according to the Standardization of

Uveitis Nomenclature (SUN) Working Group. [9] We additionally categorized patients into the following stages of uveitis duration at the time of screening: 1) onset of uveitis ≤3 months before the screening; and 2) onset of uveitis between 3 and 12 months before screening.

The results of the interferon gamma release assay (IGRA) test for tuberculosis, and serum angiotensin-converting-enzyme (ACE) levels were analyzed. A serum ACE level was considered elevated. if higher than 68 U/L.



We stopped the inclusion after 200 patients were incorporated.

FIGURE 1. Inclusion of patients. All newly referred patients with uveitis of less than 1 year duration underwent a standardized diagnostic evaluation, which included erythrocyte sedimentation rate, blood cell counts, liver and renal function studies, serum angiotensin-converting enzyme levels, serology for syphilis and Lyme disease, and chest radiographic imaging. In patients with scleritis, anterior uveitis or panuveitis, a Human Leukocyte Antigen B27 was also determined. Quantiferon testing was introduced into the diagnostic evaluation protocol in 2014, so the results were not available for all patients.

Diagnostic imaging

Clinical reports of chest radiographic findings were categorized as following: 1) consistent with sarcoidosis (i.e. symmetrical bilateral hilar lymphadenopathy and/ or suggestive interstitial lung patterns for sarcoidosis); 2) consistent with tuberculosis (TB) (i.e. asymmetrical calcified/ fibrotic lymphadenopathy); 3) indeterminite between sarcoidosis, TB or lymphoma (i.e. asymmetrical bilateral lymphadenopathy combined with either lymphadenopathy elsewhere and/ or any interstitial lung disease pattern); 4) other changes (i.e. prominent hila, vasculature changes, and chest radiographs of insufficient quality for detailed assessment resulting in a recommendation to obtain chest CT imaging); and 5) no abnormalities seen in the chest. In patients with multiple imaging investigations, the only first radiograph was classified as detailed above and included in this study.

Somatostatin receptor scintigraphy is a radionuclide scan designed to detect certain tumors

and inflammatory conditions by injection of radioactive octreotide, which binds to cells with somatostatin receptors.[10] Somatostatin receptor scintigraphy is not as widely accepted as chest CT imaging or positron emission tomography (PET) scanning for diagnosic assessment of sarcoidosis, but is considered comparable to PET imaging.[10] Somatostatin receptor scintigraphy is the first choice imaging modality when sarcoidosis is suspected at our clinic, which is recognized nationally and across Western Europe as a sarcoidosis referral and research center.

Assessment of outcomes

Clinical suspicion of ocular sarcoidosis was defined as ocular signs suggesting evidence of sarcoid uveitis (iris nodules, large-mutton-fat keratic precipitates, sheathing along retinal veins (candle wax drippings), and/or peripheral multifocal choroiditis with vitritis). Patients who were suspected to have ocular sarcoidosis on the basis of eye examination but did not have a biopsy-confirmed etiological diagnosis were divided into two groups according to the results of chest imaging: 1) clinically presumed sarcoidosis or 2) uveitis of unknown origin. Patients were presumed to have sarcoidosis on the basis of typical findings on chest radiography, chest CT imaging, and/or somatostatin receptor scintigraphy. Patients with unexplained uveitis, normal imaging results, and elevated serum ACE levels were diagnosed as uveitis of unknown origin. A definitive diagnosis of TB-associated uveitis was based on a positive microbiology test anywhere in the body and no other explanation of uveitis.[11]

Statistical analysis

All statistical analyses were performed using SPSS software (version 22.0, Chicago, IL, USA). Continuous variables were described by mean and standard deviation, categorical variables with proportions. Sensitivities and specificities of various diagnostic tests for sarcoidosis were calculated using biopsy proven sarcoidosis patients. Biopsy proven sarcoidosis patients were categorized as true positives. For the combined sensitivity and specificity of simultaneous testing the formula according to Kanchanaraksa et al was used.[12]

Results

The final diagnoses and general characteristics of the study patients are shown in Table 1. Most of our patients had posterior segment uveitis (158/200; 79%). Slightly more were females (109/200; 55%), and a majority were Caucasian (141/200; 70%). The mean age at onset of uveitis was 47.3 (±16.7) years, and the mean age at the time of referral was 47.7 (±16.6) years.

Our study cohort of 200 subjects included 22 patients (11%) with biopsy-confirmed sarcoidosis and 12 patients (6%) with presumed sarcoidosis based on a constellation of clinical findings and abnormal imaging results (Table 2).

Chest Imaging Results

The results of screening chest radiographs are given in Table 3. An abnormal chest radiograph was found for 30 of the 200 study patients (15%). Abnormal chest radiographs were more commonly observed for non-Caucasian patients (14/59 (24%)) than for Caucasians (16/141 (11%); P=0.03).

The images were considered typical of sarcoidosis for 13 of the 30 patients (43%) with an abnormal chest radiograph. Of those 13, 11(85%) were subsequently diagnosed with biopsy-confirmed sarcoidosis and one patient was diagnosed with presumed sarcoidosis. The remaining patient had Hodgkin's disease. Radiographic suspicion of prior TB was raised for 2 of the 30 patients (7%) with an abnormal chest radiograph, but neither of those two were diagnosed with active systemic TB.

For 9 of the 30 patients (30%) with an abnormal chest radiograph, imaging abnormalities were interpreted as indeterminant between sarcoidosis and other diagnoses with potentially similar radiographic findings such as lymphoma or TB. Of these 9 patients, 3 (33%) were diagnosed with biopsy-confirmed sarcoidosis by 1 year follow-up, and 3 of the 9 (33%) were diagnosed with presumed sarcoidosis. The remaining 3 patients were diagnosed with herpes simplex virus infection, prior TB, or malignant lymphoma with intraocular and central nervous system involvement. The initial chest radiograph for the only patient with active TB revealed bilateral hilar prominence attributed initially to enlarged central vasculature, and therefore classified in the "other changes" group. Chest CT imaging for this patient revealed lymphadenopathy. Biopsy of a lymph node showed necrotizing granulomas positive for acid-fast bacteria by Ziehl-Neelsen staining.

Among the 22 patients with biopsy-confirmed sarcoidosis, 14 (64%) had an abnormal screening chest radiograph (Table 4). The chest radiograph for 11 of these 14 patients (79%)

was interpreted as typical for sarcoidosis. Only one of 12 patients with presumed sarcoidosis had a chest radiograph that was typical for sarcoidosis. Among the remaining 178 patients, 16 had an abnormal chest radiograph (9%); P<0.001).

Four of 12 patients (33%) with presumed sarcoidosis had a chest radiograph that was interpreted as typical of sarcoidosis. The other 8 patients (67%) had a normal chest radiograph. For these 8 patients, either chest CT imaging 4/8 (50%) or somatostatin receptor scintigraphy 3/8 (38%) were interpreted as compatible with a diagnosis of sarcoidosis. For one of the 8 patients (13%), both chest CT imaging and somatostatin receptor scintigraphy were compatible with sarcoidosis.

TABLE 1. Demographics of 200 patients with active uveitis and specific diagnoses made after the diagnostic procedures were completed.

	Total
Total no. of included patients	200 (100%)
Age at onset of uveitis (yrs)	
Mean (±SD)	47.3 *(±16.7)*
Age at referral (yrs)	
Mean (±SD)	47.7 *(±16.6)*
Unilateral-to-bilateral involvement	1:1.6
Male-to-female ratio	1:1.2
Race	
Caucasian	141/200 (70%)
Non-Caucasian	59/200 (30%)
Anatomical localization	
Anterior	42/200 (21%)
Intermediate	16/200 (8%)
Posterior	55/200 (28%)
Panuveitis	84/200 (42%)
Scleritis	3/200 (2%)
Cause or association with systemic disease	
Associated with systemic disease	**61/200 (31%)**
Biopsy-proven sarcoidosis	22/61 (36%)
Presumed sarcoidosis, CXR consistent	4/61 (7%)
Presumed sarcoidosis, SRS and/ or chest-CT consistent	8/61 (13%)
HLA B27-associated uveitis	10/61 (16%)
VKH-syndrome	7/61 (11%)
IBD	5/61 (8%)
Other*	5/61 (8%)

TABLE 1. Continued

	Total
Infectious	**20/200 (10%)**
Active TB-associated	1/20 (5%)
Toxoplasmosis	6/20 (30%)
Other**	13/20 (65%)
Established clinical entity	**23/200 (12%)**
BSCR	9/23 (39%)
AMPPE	3/23 (13%)
Masquerade syndrome***	9/23 (39%)
Other****	2/23 (9%)
Unknown***	**96/200 (48%)**
IGRA positive	21/96 (22%)
IGRA negative	75/96 (78%)

SD=Standard Deviation, TB= tuberculosis, CXR = chest X-ray, CT = computed tomography, SRS = somatostatin-receptor-scintigraphy, HLAB27 = human leukocyte antigen B27, VKH = Vogt- Koyanagi -Harada, IBD = inflammatory bowel disease, BSCR = birdshot chorioretinopathy, AMPPE = acute multifocal posterior placoid pigment epitheliopathy, IGRA = interferon gamma release assay.

**Includes Behçet's disease (N=2), multiple sclerosis (N=2) and arteritis temporalis (N=1).*

***Includes varicella zoster virus (N=4), rubella (N=2), cytomegalovirus (N=2), syphillis (N=2), aspergillus (N=1), bartonella (N=1), herpes simplex virus (N=1).*

****Includes lymphoma (N=6), Coats' disease (N=1), post-operative uveitis (N=1) and uveitis caused by an old retinal detachment (N=1).*

*****Includes 1 patient with sympathetic ophthalmia and 1 patient with Fuchs heterochromic uveitis syndrome (FHUS)*

******Includes 1 patient with uveitis suspected to be caused by Bacillus Calmette Guerin intravesical immunotherapy for bladder cancer.*

No difference in the fraction of patients with an abnormal chest radiograph was found between those with onset of uveitis less that <3 months prior to our evaluation (24/142 (17%) versus those with onset between 3 and 12 months earlier 6/58 (10%); P=0.24).

The fraction of subjects with abnormal chest radiographs was similar for patients with anterior uveitis (5/42 (12%)) and for those with non-anterior uveitis (25/158 (16%); P=0.81). Biopsy-confirmed sarcoidosis was more common among patients with panuveitis (17/84 (20%)) compared to patients with another anatomical location of uveitis (5/116 (4%); P<0.001). The majority of patients with biopsy-confirmed sarcoidosis and panuveitis had vitritis (13/22; 59%). Among those 13 patients, 9 (69%) had vitritis combined with peripheral multifocal choroiditis. Presumed sarcoidosis was more prevalent among patients with panuveitis (10/84 (12%)) than among patients who did not have panuveitis 2/116 (2%); P=0.003). Most patients had vitritis (10/12 (83%). Of those 10 pateints, 4(40%) had vitritis combined with peripheral multifocal choroiditis.

TABLE 2. Characteristics of patients with uveitis and biopsy -proven or presumed sarcoidosis.

	Biopsy proven sarcoidosis	Presumed sarcoidosis		Remainder of patients
		Biopsy negative or chest radiograph positive	***Biopsy negative or not done; CT or SRS positive***	
Total no. of patients	22	4	8	166
Anterior uveitis	3/22 (14%)	1/4 (25%)	0	38/166 (23%)
Panuveitis	17/22 (77%)	3/4 (75%)	7/8 (8%)	57/166 (34%)
Age at onset				
Mean years (±SD)	47.2 (±15.9)	51.0 (±20.2)	59.5 (±19.8)	46.5 (±16.4)
Male-to-female ratio	1:2.1	1:1	1:1.6	1:0.9
Non-Caucasian	2/22 (9%)	3/4 (75%)	1/8 (13%)	53/166 (32%)
Abnormal Chest Radiograph	14/22 (64%)	4/4 (100%)	0	12/166 (7%)
Chest CT available	13/22 (59%)	0	5/8 (63%)	27/166 (16%)
Abnormal	12/13 (92%)	0	5/5 (100%)	6/27 (22%)
SRS available	9/22 (41%)	0	4/8 (50%)	27/166 (16%)
Abnormal	7/9 (78%)	0	4/4 (100%)	3/27 (11%)
ACE available	22/22 (100%)	4/4 (100%)	8/8 (100%)	150/166 (90%)
Elevated	9/22 (41%)	3/4 (75%)	3/8 (38%)	6/150 (4%)

CT = computed tomography, SRS = somatostatin receptor scintigraphy, ACE = Angiotensin-Converting-Enzyme.

TABLE 3. Results of chest radiographs.

	Total N=200
Abnormal chest radiograph	30/200 (15%)
Most probable chest radiologic diagnosis	
Sarcoidosis	13/30 (43%)
Tuberculosis	2/30 (7%)
Indeterminate between Sarcoidosis, Tuberculosis and lymphoma	9/30 (30%)
*Other changes**	6/30 (20%)

**The chest radiograph of the only patient with active tuberculosis was classified into this group.*

TABLE 4. Review of chest radiography results in the sarcoidosis population.

	Biopsy proven sarcoidosis N=22	Presumed sarcoidosis N=12
Abnormal chest radiograph	**14/22 (64%)**	**4/12 (33%)**
Most probable radiologic diagnosis		
Sarcoidosis	11/14 (79%)	1/4 (25%)
Tuberculosis	0	0
Indeterminate between Sarcoidosis, Tuberculosis and lymphoma	3/14 (21%)	3/4 (75%)
Other changes	0	0

Most patients with biopsy-confirmed sarcoidosis had a chest CT scan that showed finding interpreted as typical for sarcoidosis (12/13 (92%) versus 11/32; 34%; P<0.001) as was a positive somatostatin receptor scintigraphy (7/9; 78% versus 7/31; 23%; P=0.004).

Blood test results

Serum ACE measurements were available in 184 patients, of whom 21/184 (11%) exhibited an elevated level. (Table 3). Serum ACE levels were more frequently elevated in the biopsy-proven sarcoidosis group compared to the remaining patients (9/22; 41% versus12/162; 7%, P<0.001). Serum ACE levels were abnormally elevated for 6 of 12 patients (50%) with presumed sarcoidosis. The combined sensitivity and specificity of serum ACE levels and chest radiography for the diagnosis of biopsy-proven sarcoidosis was 79% and 84% respectively (Table 5).

A Quantiferon-TB Gold test was performed on 126/200 (63%) of patients and was found positive for 23 patients (18%). The fraction of abnormal chest radiographs was similar for the Quantiferon positive (4/23 (17%)) and the Quantiferon negative groups (15/103 (15%); P=0.750).

Sensitivity and specificity of diagnostic studies

The sensitivity and specificity of various diagnostic modalities for biopsy-confirmed sarcoidosis is shown in Table 5. The sensitivity and the specificity of a screening chest radiograph interpreted as typical for sarcoidosis was 64% and 91% respectively for biopsy-confirmed disease.

TABLE 5. Sensitivity and specificity of different imaging modalities and serum angiotensin converting enzyme for biopsy-proven sarcoidosis (N=22).

	Sensitivity (95% CI)	**Specificity (95%CI)**
Chest radiograph*	64% (43.0-80.3)	91% (85.9-94.4)
Chest CT**	94% (85.9-94.4)	89% (71.9-96.1)
SRS***	78% (45.3-93.7)	77% (59.1-88.2)
Serum ACE****	41% (23.3-61.3)	93% (87.5-95.7)

CT = computed tomography, SRS = somatostatin-receptor-scintigraphy, CI=Confidence Interval, ACE angiotensin converting enzyme.

**Chest racdiographs were available for 200 of 200 (100%) patients.*

***Chest CT imaging was obtained for 45 patients who were suspected of sarcoidosis (45% of the total patient cohort).*

****SRS was obtained for 40 patients who were suspected of sarcoidosis (20% of the total patient cohort).*

*****Serum ACE was available in 184/200 (92%) of patients; in 6 patients serum ACE was not determined (for non-medical reasons).*

Discussion

The fraction of patients with uveitis that is attributed to ocular sarcoidosis in Western Europe and North America ranges from 3 to 17%.[10, 13-17] However, the diagnostic value of chest radiography for patients with uveitis of unknown origin remains obscure. In this observational study from an ophthalmology tertiary-level referral center, 15% of 200 patients with active uveitis of onset within the previous 12 months were found to have an abnormal chest radiograph. A large proportion of those were interpreted as compatible with the diagnosis of sarcoidosis.

To our knowledge, the only previous study specifically addressing the utility of chest radiography for the etiologic diagnosis of uveitis was published in 1988.[18] The authors of that study reported that 0.5% (4/758) of screening chest radiographs were interpreted as consistent with a diagnosis of sarcoidosis, compared to 15% for our study cohort. This difference may largely be explained by the high proportion of patients with anterior uveitis (72%) in the previous study, which is typical for a primary or secondary ophthalmology practice. Tertiary centers usually treat more severe uveitis patients such that the proportion of patients with anterior uveitis is typically 20 to 25%, as was observed for our study cohort.[19-22] Moreover, in contrast to 0.5% for the 1988 studt, the fraction of patients with uveitis was closer to 10% in recent reports from similar geographic areas.[23]

Most other previous studies reporting chest radiographic findings for patients with uveitis were performed on patients with biopsy-confirmed or presumed sarcoidosis and thus do not address the value of screening with chest radiographs.[24-27] These studies show a wide variation in percentages of patients with abnormal chest radiographs, ranging from 35 to 94%.[24, 25, 28] These percentages vary due to selection of patients and/or radiologic images included in the studies. Chest radiographs have been shown to be positive for more than 90% of patients with sarcoidosis at some point in the evolution of the diseased.[27] Therefore, the time of performing chest radiography might influence the percentage of positive results. Also, most previously reported studies did not specify whether the first chest radiograph obtained after onset of uveitis or a selected radiograph from among several obtained over time was selected for analysis.

In contrast, we present a homogenous cohort of patients with active uveitis of limited duration and undetermined cause, and we include solely the first chest radiograph performed after onset of uveitis. In our series, 14 of 22 patients (64%) with biopsy-confirmed sarcoidosis of onset within 1 year prior to our assessment exhibited abnormalities on a screening chest radiograph and 11 of those 14 (79%) had findings that were interpreted as typical for sarcoidosis.

The causes and prevalence of specific systemic associations for uveitis vary worldwide due to geographical and environmental differences.[22] The prevalence of ocular sarcoidosis also shows a strong geographical variation.[22] In addition, the type of medical center (primary, secondary or tertiary) plays a role in the selection of patients. Consequently, the results of our study, performed at a tertiary center in Western Europe, may not be generalizable to primary and secondary care centers or to other regions of the world.

In our study, the sensitivity and specificity of chest CT imaging and somatostatin receptor scintigraphy were much higher than for chest radiography. It should be kept in mind that the patients who underwent chest CT scanning or somatostatin receptor scintigraphy were selected either on clinical grounds or because nondiagnostic abnormalities were seen on the screening chest radiograph. Therefore, the sensitivity and specificity for CT imaging and somatostatin receptor scintigraphy derived from our study results cannot be used for calculations in an unselected uveitis population.

The question whether it is better to omit screening chest radiography from the initial evaluation of patients with uveitis and use more sensitive imaging techniques only in patients with suspected sarcoidosis based on clinical, laboratory and/or clinical grounds, cannot be answered by our study. To make this decision, the sensitivity and specificity of all employed diagnostic methods should be known. To determine the relative sensitivities and specificities of these investigations, simultaneous imaging by chest radiography, chest CT imaging and somatostatin receptor scintigraphy would be required.

In summary, our study shows that an abnormal chest radiograph was observed for 15% of 200 patients with active uveitis of unknown origin of and of less than one-year duration. The most common radiologic diagnosis was sarcoidosis.

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chapter 33

Diagnostic Value of Serum-Soluble Interleukin 2 Receptor Levels vs Angiotensin-Converting Enzyme in Patients With Sarcoidosis-Associated Uveitis

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JAMA Ophthalmol. 2017 Dec 1;135(12):1352-1358.

Abstract

Importance New and improved diagnostic tests for sarcoidosis-associated uveitis are needed because the currently available laboratory diagnostic biomarkers (e.g. lysozyme and angiotensin-converting enzyme (ACE)) are lacking in high sensitivity and specificity.

Objective To compare the value of soluble IL-2 Receptor (sIL-2R) with ACE as diagnostic biomarkers of sarcoidosis in patients with uveitis.

Design, Setting and Participants A cross-sectional retrospective study was conducted using data collected from 249 consecutive patients with uveitis at the Erasmus University Medical Center outpatient clinic, Rotterdam, The Netherlands, from April 3, 2013, through November 25, 2015. Measurements of sIL-2R and ACE in serum samples and data extraction from patient files were conducted from December 2016 through February 2017, and analysis from April to May 2017.

Main Outcomes and Measures Serum concentrations of sIL-2R and ACE and chest radiography outcomes were assessed. Receiver operating characteristics analysis was used to determine the probability that individual tests correctly identified patients with sarcoidosis. The Youden Index was used to determine the optimal cutoff points for serum sIL-2R and ACE levels to define sarcoidosis in patients with uveitis.

Results Data were analyzed from 249 patients with uveitis who had their serum sIL-2R and ACE levels determined and underwent chest radiography. Mean (SD) age at the time of sampling was 51 (16) years, 161 (64.7%) were women, and 191 (76.7%) were white. Although patients with sarcoidosis-associated uveitis had the highest mean serum sIL-2R (6047 ±2533 pg/mL) and ACE (61 ±38 U/L) levels, elevated serum sIL-2R levels were also found in patients with HLA-B27-associated (4460 ±2465 pg/mL) and varicella-zoster virus-associated (5386 ±1778 pg/mL) uveitis. Serum sIL-2R and ACE levels were significantly correlated (Pearson correlation coefficient, 0.205; P=.001, 2-sided), but no association was found between uveitis activity and sIL-2R (Spearman rank correlation coefficient ρ, 0.070, P=.27) nor uveitis activity and ACE (ρ, -0.071; P=.27). The highest Youden index for sIL-2R alone was 0.45, corresponding to an optimal cutoff of 4000 pg/mL and providing 81% (95% CI, 74%-89%) sensitivity and 64% (95% CI, 56%-72%) specificity alone but combined with chest radiography yielded 92% sensitivity and 58% specificity. Chest radiography combined with sIL-2R at a cutoff of 6000 pg/mL resulted in 77% sensitivity and 73% specificity. Combined chest radiography and serum ACE levels at the standard cutoff of 68 U/L resulted in 70% sensitivity and 79% specificity.

Conclusion and relevance This cross-sectional study demonstrates that sIL-2R is a useful marker for diagnosing sarcoidosis in patients with uveitis and has slightly better diagnostic value than ACE.

Introduction

Sarcoidosis is a major causes of uveitis worldwide.[1] An accurate diagnosis of sarcoidosis in patients with uveitis has consequences for the management of the patients' care and their vision outcomes as well as the choice of medication. Determining whether a patient with uveitis also has sarcoidosis is usually assessed using chest imaging in combination with biochemical measures and is preferably confirmed by biopsy results.[2]

The lack of a highly sensitive and specific screening test for sarcoidosis in patients with uveitis poses a substantial problem for diagnosing sarcoidosis, because undetected sarcoidosis can lead to substantial systemic and ocular morbidity.[3] Although serum angiotensin converting enzyme (ACE) is the most commonly used diagnostic and activity marker for sarcoidosis, this biomarker has low sensitivity.[4-6]

The soluble IL-2 receptor (sIL-2R; also termed CD25) is a truncated protein that is released from activated T-cells; hence, it is a surrogate marker for T-cell activation.[7] Activation of T-cells is a main component of the inflammatory process in sarcoidosis, and sIL-2R serum levels indeed correlate with disease activity in sarcoidosis.[8-12] However, sIL-2R has been scarcely investigated in sarcoidosis-associated uveitis, and its diagnostic value in patients with uveitis is not clear.[5]

We assessed the value of sIL-2R as a diagnostic biomarker for sarcoidosis-associated uveitis, determining its sensitivity and specificity and comparing these results with those of ACE.

Methods

Study Population

We conducted a cross-sectional study in consecutive patients with uveitis, who visited the Ophthalmology Department at the Erasmus University Medical Center, Rotterdam, the Netherlands. All participating patients visited the department from April 3, 2013, through November 25, 2015, for evaluation, treatment, or both and agreed to have samples included in a biobank for use in research studies. The study was designed in November 2016. Measurements of sIL-2R and ACE in serum samples were conducted from December 2016 through February 2017. Data from patient files were abstracted between December 2016 through April 2017. Data analysis was performed from April to May 2017.

The medical ethical committee of Erasmus University Medical Center approved the biobanking protocol and the associated procedures. Written informed consent was obtained for use of the biobank material, which adheres to the tenets of the Declaration of Helsinki.

Assessment of clinical characteristics

Demographic data as well as the final diagnosis of uveitis and its onset, laterality, and location were recorded. Uveitis onset was defined as the date on which an ophthalmologist first documented uveitis. At the time of sampling, 152 of 249 patients (61.0%) had active uveitis. Use of immunosuppressive medications and ACE-inhibitors were also recorded.

Assessment of Serum sIL-2R and ACE levels

For missing routine diagnostic ACE or sIL-2R results, serum levels were determined with biobank samples (stored at -80°C) using the same standard diagnostic facilities and laboratory methods. For all patients, ACE and sIL-2R levels were measured in all serum samples on the same day.

Serum sIL-2R and ACE level assessments were performed by a laboratory with an ISO15189:2012 accreditation. Both assays were within the scope of this certification and as such are subjected to periodic external quality assessment.

Serum sIL-2R levels were determined using an enzyme-linked immunosorbent assay (Human sCD25/sIL-2R ELISA kit, Diaclone, Besancon Cedex, France) according to manufacturer instructions. The interassay variation coefficient for the sIL-2R measurement was 12%. Freeze-thaw cycles of samples did not affect sIL-2R values up until the third cycle. In addition, material could be stored at room temperature for 3 days without affecting the sIL-2R values. A value greater than 2500 pg/mL indicated a level that was elevated compared with that in a healthy population.

The interassay coefficient of variation for ACE, used as an internal quality control, was 2.2% at 46 U/L and 2.1% at 84 U/L (to convert ACE levels to nanokatals per liter, multiply by 16.667). This was below the manufacturers claim of 8.1% for interassay precision. The influence of freeze-thaw cycles on ACE was not investigated.

The ACE levels were determined using a commercial ACE kinetic assay kit (Bühlmann laboratories AG, Switzerland), which has a CE (Conformité Européenne) marking, analysed spectrophotometrically on an automated analyzer (Cobas 8000; Roche Diagnostics). The assay is based on the enzymatic cleavage by ACE of the synthetic substrate FAPGG (*N*-[3-(2-16 furyl)acryloyl]-L-phenylalanyl-L-glycyl-L-glycine) into an amino acid derivative and dipeptide. The kinetics of this reaction was measured by detecting the decrease in absorbance at a wavelength of 340 nm. The standard cutoff for serum ACE levels of greater than 68 U/mL was used.

Chest Imaging Assessment

Chest radiography had been conducted in 190 of 249 participants (76.3%). When multiple images were available, the radiograph dated closest to that of serum sampling was selected. However, chest radiograph had to have been performed within 12 months (before or after) of blood sampling to be included in our analyses. Radiographic signs consistent with the diagnosis of sarcoidosis were symmetrical bilateral hilar lymphadenopathy and/or interstitial lung patterns suggestive of sarcoidosis or both. All other changes were classified according to current radiologic criteria.

Outcome assessment

Only patients with definitive or presumed ocular sarcoidosis based on the International Workshop on Ocular Sarcoidosis criteria were included (biopsy and/or radiologic finding), and patients with probable or possible ocular sarcoidosis were classified as having unknown origin.[2] A definite diagnosis of tuberculosis-associated uveitis was made in patients with a positive microbiology test result in samples obtained anywhere in the body without another explanation of uveitis.[13] Other diagnoses were made based on current international criteria.[14-19] Active uveitis was defined as the presence of anterior chamber cells or vitreous cells, opalescent anterior chamber, or vasculitis or retinitis as documented by ophthalmoscopy or fluorescence angiography.

Statistical analysis

Patient characteristics were summarized using descriptive statistics, including means (SDs) and percentages. Unpaired *t* tests were used to compare characteristics between the groups. A 2-sided $P<.05$ was considered statistically significant. The test characteristics for

sIL-2R as well as for ACE in the diagnosis of sarcoidosis (ie, sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) were calculated. Receiver operating characteristic (ROC) curves were plotted, and the C statistics (ie, the area under the ROC curve) for sIL-2R and for ACE were calculated. The ROC curve is a plot that shows the sensitivity and specificity of a test at all possible cutoff values that could be used to distinguish patients anticipated to have a disease from those who do not. The sensitivity and specificity of a test at all possible cutoff values that could be used to distinguish patients anticipated to have a disease from those who do not. The sensitivity and specificity are calculated at every observed value in the data set and are plotted to form the ROC curve. The area under this curve, termed the *C statistic*, describes the probability that the test will correctly identify patients with the disease and can vary between 1 (perfect sensitivity and specificity) and 0.5 (no better than chance and thus a useless test).

The sensitivity and specificity for the use of chest radiography in the diagnosis of sarcoidosis was also determined. In addition, the Youden indices (J = sensitivity + [specificity - 1]) for sIL-2R, ACE, and chest radiographic results were calculated. This index, which ranges from -1 to 1, indicates that the diagnostic test is useless when it equals zero because this would mean the same proportion of positive results were obtained for groups with and without the disease. A higher Youden index is more favourable because a value of 1 indicates no false-positives or false-negatives.[20] The Youden index in the ROC curve analysis was used to determine the optimal cutoff levels for sIL-2R and ACE. Combined sensitivity and specificity were also calculated using the method for simultaneous testing according to Kanchanaraksa et al.[21] The statistical analyses were conducted using Excel; IBM SPSS Statistics for Windows, version 21.0.0 (IBM Crop); and R, using the software package pROC.

Results

Patient inclusion is illustrated in the study flowchart (Figure 1). From April 3, 2013, through November 25, 2015, 266 patients with uveitis agreed to participate in our biobank study, of which 249 had their serum sIL-2R and ACE levels simultaneously measured.

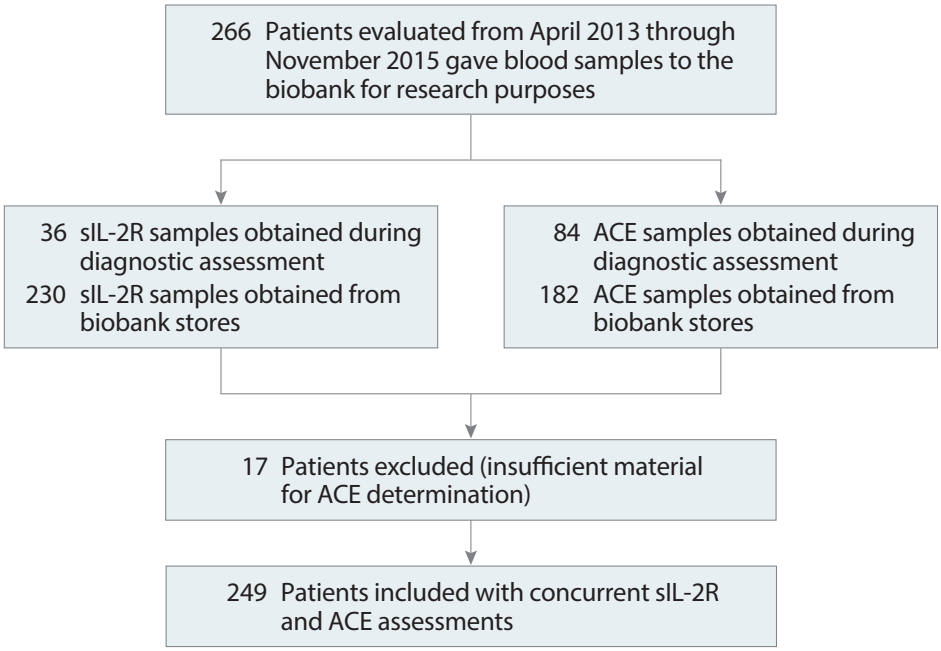

FIGURE 1. Flow Diagram of Patients and Samples Included in the Study ACE indicates angiotensin-converting enzyme; sIL-2R, soluble interleukin 2 receptor.

Population characteristics

Final diagnoses and demographic characteristics of the study cohort are given in Table 1. The mean (SD) age at uveitis onset was 46 (17) years and at sampling for the present study was 51 (16) years. Women had significantly higher mean (SD) ACE levels than men (49 [28] versus 39 [24]; P=.01), whereas serum sIL-2R levels were similar between the sexes (women, 4070 [2224] versus 4509 [2490] pg/mL; P=.16). Age and serum sIL-2R showed a significant linear association (P = 0.045): serum sIL-2R increased approximately 18 pg/ mL every year. The results of serum sIL-2R levels are illustrated in Figure 2 and given in Table 1. The mean (SD) sIL-2R level of patients with uveitis associated with systemic noninfectious disease was 4823 [2502] pg/mL and in patients with infectious uveitis was 4268 [2011] pg/mL. Within the systemic disease group, the highest mean (SD) serum sIL-2R (6047 [2533] pg/mL) as well as

serum ACE (61 [38]) levels were noted in patients with sarcoidosis. Patients with HLA-B27-associated uveitis also exhibited high mean (SD) sIL-2R values (4460 [2465] pg/mL; P=.08 compared with patients with sarcoidosis). Within the samples obtained from patients with infectious uveitis, varicella-zoster virus-associated uveitis had the highest mean (SD) sIL-2R (5386 [1778] pg/mL and ACE (50 [26] U/L) serum levels. Low mean (SD) sIL-2R levels were found in patients with birdshot chorioretinopathy (2980 [1174] pg/mL;*P* <.05 compared with patients with sarcoidosis). Mean sIL-2R serum levels did not differ between those patients using any form of systemic immunosuppressant therapy and those who did not (for the whole population: 3872 [2456] pg/mL vs 4422 [2235] pg/mL, P=.91; for patients with sarcoidosis: 5450 [2955] pg/mL vs 6333 [2314] pg/mL; P=.33).

TABLE 1. Soluble Interleukin-2 Receptor and Angiotensin Converting Enzyme in patients with uveitis.

	No	**Serum ACE** Mean *(±SD)* U/mL	**Serum sIL-2R** Mean *(±SD)* pg/mL
Total no. of included patients	249 (100%)	46 (±27)	4225 (±2326)
Age at onset of uveitis (yrs) Mean *(±SD)*	46 *(±17)*	NA	NA
Age sampling (yrs) Mean *(±SD)*	51 *(±16)*	NA	NA
Unilateral involvement	87/249 (35%)	39 (±22)	4212 (±2432)
Bilateral involvement	162/249 (65%)	49 (±28)	4233 (±2275)
Males	88/249 (35%)	39 (±24)	4509 (±2490)
Females	161/249 (65%)	49 (±28)	4070 (±2224)
Race			
Caucasian	*191/249 (77%)*	47 (±28)	4198 (±2218)
Non-Caucasian	*58/249 (23%)*	41 (±22)	4316 (±2672)
Anatomical localization of uveitis			
Anterior	*37/249 (15%)*	44 (±23)	4373 (±2585)
Intermediate	*21/249 (8%)*	43 (±20)	4236 (±2086)
Posterior	*77/249 (31%)*	47 (±25)	3848 (±2230)
Panuveitis	*103/249 (41%)*	47 (±32)	4554 (±2416)
Scleritis	*11/249 (4%)*	36 (±17)	3257 (±918)
Use of medication			
Immunosuppressive medication	*89/249 (36%)*	47 (±27)	3872 (±2456)
ACE-inhibitor	*16/249 (6%)*	46 (±27)	4628 (±1861)
Activity of uveitis			
Active uveitis	*152/249 (61%)*	46 (±28)	4430 (±2474)
Remission of uveitis	*97/249 (39%)*	45 (±25)	3905 (±2045)

TABLE 1. Continued.

	No	Serum ACE Mean *(±SD)* U/mL	Serum sIL-2R Mean *(±SD)* pg/mL
Associated with systemic disease	**77/249 (31%)**	**48 (±32)**	**4823 (±2502)**
Sarcoidosis, total	37/77 (48%)	*61 (±38)*	*6047 (±2533)*
Definitive sarcoidosis	*23/77 (30%)*	*55 (±27)*	*5521 (±2232)*
Presumed sarcoidosis	*14/77 (18%)*	*70 (±51)*	*6911 (±2835)*
Multiple sclerosis	*13/77 (17%)*	*37 (±18)*	*3487 (±1431)*
HLA B27-associated uveitis	*10/77 (13%)*	*37 (±23)*	*4460 (±2465)*
VKH- syndrome	*4/77 (5%)*	*28 (±11)*	*2834 (±1694)*
Miscellaneous[a]	*13/77 (17%)*	*35 (±22)*	*3569 (±1847)*
Infectious uveitis	**50/249 (20%)**	**37 (±19)**	**4268 (±2011)**
Rubella virus	*16/50 (32%)*	*30 (±14)*	*3915 (±1885)*
Toxoplasmosis	*12/50 (24%)*	*39 (±21)*	*3378 (±1654)*
Cytomegalovirus	*7/50 (14%)*	*37 (±23)*	*4537 (±1611)*
Varicella-zoster virus	*7/50 (14%)*	*50 (±26)*	*5386 (±1778)*
Miscellaneous[b]	*8/50 (16%)*	*36 (±15)*	*5092 (±2780)*
Established clinical entity	**47/249 (19%)**	**44 (±23)**	**3738 (±2442)**
BSCR	*25/47 (53%)*	*49 (±26)*	*2980 (±1174)*
Masquerade syndrome[c]	*9/47 (19%)*	*43 (±22)*	*5741 (±3701)*
AMPPE	*3/47 (6%*	*38 (±13)*	*5184 (±4180)*
Miscellaneous[d]	*10/47 (21%)*	*38 (±16)*	*3395 (±2076)*
Unknown[e]	**75/249 (30%)**	**50 (±27)**	**3889 (±2163)**
IGRA positive	*20/75 (27%)*	*50 (±30)*	*4147 (±2292)*
IGRA negative or not performed	*55/75 (73%)*	*50 (±25)*	*3717 (±2081)*

sIL-2R = soluble interleukin 2 receptor, ACE= angiotensin converting enzyme, NA=not applicable, HLAB27 = human leukocyte antigen B27, VKH = Vogt- Koyanagi -Harada, IBD = inflammatory bowel disease, BSCR = birdshot chorioretinopathy, AMPPE = acute multifocal posterior placoid pigment epitheliopathy, FHUS = Fuchs Heterochromic Uveitis Syndrome, BSCR = birdshot chorioretinopathy, VZV = Varicella Zoster Virus, CMV= Cytomegalovirus, HLAB27 = human leukocyte antigen B27, IGRA = Interferon Gamma Release Assay.

[a] Including granulomatosis with polyangiitis (N=3), juvenile idiopathic arthritis (N=2), Sjögren's disease (N=1), inflammatory bowel disease (N=1), polychondritis (N=1), morphea (N=1), Kikuchi disease (N=1), Bechet's disease (N=1), ankylosing spondylitis (N=1), giant-cell arteritis (N=1).

[b] Including herpes –simplex virus (N=3), human immunodeficiency virus (N=1), tuberculosis (N=1), styphylococcus aureus (N=1), streptococcus pneumoniae (N=1), aspergillus niger (N=1).

[c]Including lymphoma (N=7), retinitis pigmentosa (N=1), uveitis suspected to be caused by bacillus calmette guerin intravesical immunotherapy for bladder cancer (N=1).

[d] Including Fuchs Heterochromic Uveitis Syndrome (N=3), Ampiginous Choroiditis (N=2), Sympathic Ophthalmia (N=2), Acute Zonal Occult Outer Retinopathy (N=1), Presumed Ocular Histoplasmosis Syndrome (N=1) and post-traumatic uveitis (N=1).

[e] In 10 patients with uveitis of unknown cause, no IGRA test was performed.

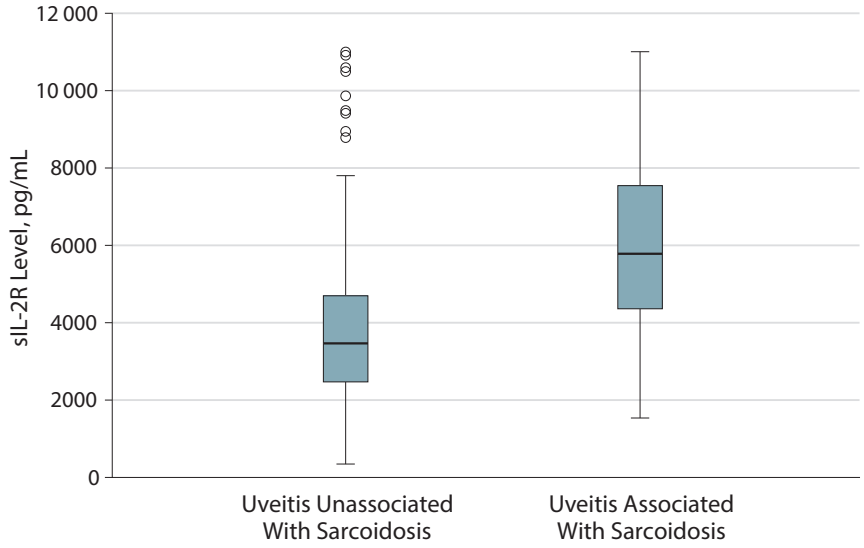

FIGURE 2. Plot Comparing Soluble Interleukin 2 Receptor (sIL-2R) Serum Levels in Patients With Sarcoidosis-Associated Uveitis and With Uveitis Unassociated With Sarcoidosis.

Boxes indicate the interquartile range; bold horizontal lines, medians; whiskers, the minimum and maximum, excluding the outliers; and open circles, outliers.

Determining the Optimal Cutoff for Serum sIL-2R and ACE Levels to Define Sarcoidosis-Associated uveitis

Use of a sIL-2R cutoff value of 2500 pg/mL resulted in a relatively low Youden index of 0.17 (Table 2). Therefore, to calculate the optimal cutoff for the diagnosis of sarcoidosis-associated uveitis, we maximized the Youden index in our ROC curve. The highest Youden index for sIL-2R was 0.45, which yielded an optimal cutoff of 4000 pg/mL. Corresponding sensitivity for the diagnosis of sarcoidosis was 81% (95% CI, 65%-92%), and the corresponding specificity was 64% (95% CI, 57%-72%). To ensure a fair comparison of sIL-2R and ACE levels, we also calculated an optimal cutoff point for serum ACE levels in the population with uveitis. The optimal cutoff point for ACE was 51 U/mL, which was marginally lower than the currently used standard cutoff 68 U/mL.

Evaluating the Value of the Various Diagnostic Tests for Sarcoidosis-Associated Uveitis

Table 2 provides the results of the various diagnostic tests using the reference values and optimized cutoffs. The Youden index (with optimized cut-offs) was higher for sIL-2R than for ACE levels (0.45 versus 0.23). In addition, the C statistic (area under the ROC curve) also favored sIL-2R over ACE (0.76 [95%CI, 0.68-0.84] vs 0.65 [95%CI, 0.55-0.74]; P=.06, 2-sided, DeLong test; P < .05 considered statistically significant) (Figure 3). We compared different

TABLE 2. Sensitivity, specificity, Youden's J-statistic, C-statistic positive and negative predictive values of diverse diagnostic tests for uveitis associated with sarcoidosis.

	Sensitivity (95% CI)	Specificity (95% CI)	Youden's J statistic	C-statistic (95% CI)	PPV	NPV
sIL-2R ≥ *2500 pg/mL*	92% (78-98%)	26% (20-32%)	0.17	0.76 (0.68-0.84)	0.18	0.95
sIL-2R ≥ *4000 pg/mL*	81% (65-92%)	64% (57-70%)	0.45	0.76 (0.68-0.84)	0.28	0.95
sIL-2R ≥ *6000 pg/mL*	47% (31-63%)	79% (73-86%)	0.27	0.76 (0.68-0.84)	0.28	0.90
ACE ≥*68 U/mL*	30% (16-47%)	85% (80-90%)	0.15	0.65 (0.55-0.74)	0.26	0.87
ACE ≥*51 U/mL*	54% (37-71%)	70% (63-76%)	0.23	0.65 (0.55-0.74)	0.24	0.90
Chest Radiograph [a]	56% (38-73%)	98% (94-100%)	0.48	NA	0.83	0.93
ACE ≥*68 U/mL* + **chest radiograph** [a, b]	70% (NA)	79% (NA)	0.49	NA	0.42	0.92
ACE ≥*51 U/mL* + **chest radiograph** [a, b]	82% (NA)	64% (NA)	0.45	NA	0.33	0.94
ACE ≥*68 U/mL* + **sIL-2R** ≥ *4000 pg/mL* [b]	87% (NA)	55% (NA)	0.42	NA	0.30	0.95
ACE ≥*51 U/mL* + **sIL-2R** ≥ *4000 pg/mL* [b]	92% (NA)	44% (NA)	0.36	NA	0.26	0.96
sIL-2R ≥ *4000 pg/mL* + **chest radiograph** [a, b]	92% (NA)	58% (NA)	0.50	NA	0.32	0.97
sIL-2R ≥ *6000 pg/mL* + **chest radiograph** [a, b]	77% (NA)	73% (NA)	0.50	NA	0.38	0.94
sIL-2R ≥*3195 pg/mL Current study*	92% (78-98%)	43% (36-50%)	0.35	NA	0.20	0.96
sIL-2R ≥*3195 pg/mL Gundlach et al* [c]	98% (87-100%)	94% (90-97%)	0.92	NA	NA	NA

sIL-2R = soluble interleukin 2 receptor, ACE= angiotensin converting enzyme, NA=Not Applicable or Not Available, cannot be calculated for combination of tests, PPV= positive predictive value.
NPV=negative predictive value
[a] *In total, 190/249 (76%) underwent chest radiography. Out of all, 37 (19.5%) patients did not have a chest radiograph for diagnostic purposes of uveitis screening, but for follow-up of uveitis (N=9) or for any other medical reasons than uveitis (N=28). Only sarcoidosis signs, such as lymphadenopathy or interstitial lung patterns, were seen as signs of sarcoidosis.*
[b] *To calculate the combined sensitivity and specificity of the various combinations of sIL-2R, ACE and chest radiograph, we used the sensitivity and specificity of sIL-2R, ACE and chest radiograph calculated in a sub-cohort of 190 patients who underwent all three tests of whom 18% (34/190) had sarcoidosis.*
[c] *In this study, however, also patients without histologic or radiologic evidence of sarcoidosis were included and thus a part of included patients was based on elevated sIL-2R and ACE levels, see reference 5). We assumed a conversion factor for sIL-2R of 5.*

diagnostic strategy combinations by using the Youden index (Table 2). The combination that yielded the highest Youden index, was sIL-2R and chest radiography. The cutoffs for sIL-2R levels of at least 4000 pg/mL and at least 6000 pg/mL both resulted in a Youden index of 0.50. The cut-off of 4000 pg/mL or greater corresponded to a sensitivity of 92% and specificity of 58%, whereas the cutoff of 6000 pg/mL or greater resulted in a more balanced sensitivity of 77% and specificity of 73%.

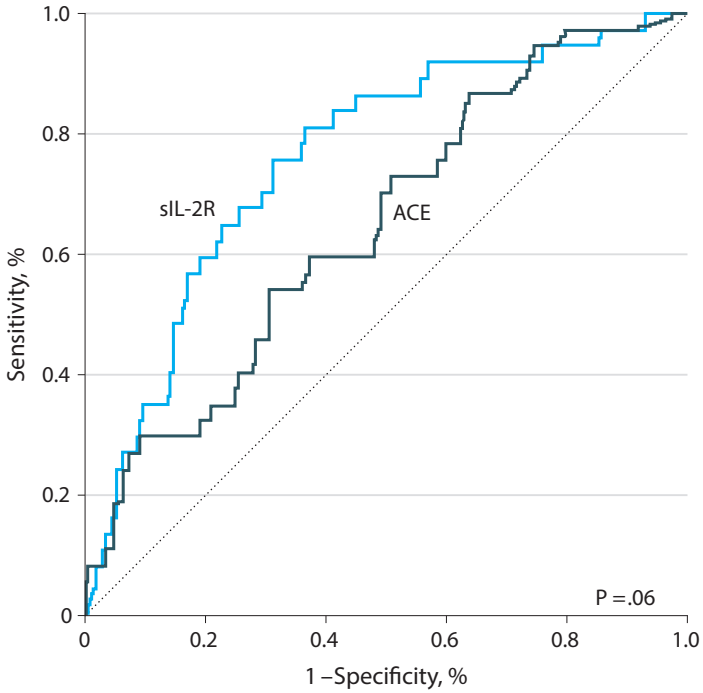

FIGURE 3. Receiver Operating Characteristic Curves Comparing Soluble Interleukin 2 Receptor (sIL-2R) and Angiotensin-Converting Enzyme (ACE) levels

Area under the curve for sIL-2R levels is 0.76 (95% CI, 0.68-0.84); for ACE levels, 0.65 (95% CI, 0.55-0.74) (P=0.06, 2-sided, Delong test; P < 0.05 considered statistically significant).

The combination of ACE (standard cutoff) and chest radiography yielded a sensitivity of 70% and specificity of 79% and a Youden index of 0.49. We also calculated the PPV and the NPV for the various diagnostics tests and combinations. Both PPV and NPV favored sIL-2R levels with a cutoff equal to or greater than 4000 pg/mL over ACE with a cutoff equal to or greater than 51 U/mL, with values of PPV and NPV of 0.28 and 0.95, respectively, for sIL-2R and of 0.24 and 0.90, respectively, for ACE.

In addition to these data, we also examined a cohort that excluded all patients who used any therapy that might have influenced the outcomes of the sIL-2R or ACE assays (ie, any systemic immunomodulatory therapy or ACE inhibitor therapy). In this cohort of 157 patients (including 24 patients [15.3%] with sarcoidosis), we calculated the sensitivity, specificity, and the C statistic for the serum sIL-2R and ACE levels. The results for the original cohort of 249 participants and this smaller cohort of 157 did not differ for the sIL-2R assay, but the sensitivity of the ACE assay increased from 54% to 71% in the smaller cohort. The C statistic for sIL-2R and for ACE did not differ (0.80 vs 0.73*; P* = .27, 2-sided, Delong test) and was similar to the results for the original cohort of 249 patients (0.76 vs 0.65; *P* = .06; 2-sided, Delong test).

Serum sIL-2R and ACE Levels and Uveitis Activity

A positive correlation was observed between serum sIL-2R and ACE levels (Pearson correlation coefficient, 0.205; P=.001, 2-sided). No correlation between uveitis activity and sIL-2R or ACE levels was observed for the whole study population (Spearman's rho, 0.070, P=.27 vs -0.071, P=.27) or for only those patients with sarcoidosis (Spearman's rho, 0.260, P=.12 vs 0.127, P=.45, respectively).

Discussion

This cross-sectional study revealed that the level of sIL-2R was slightly better than that of ACE in its diagnostic performance of sarcoidosis in a population of patients with uveitis. Serum sIL-2R levels also showed slightly better C statistic outcomes and had a slightly higher Youden index than for ACE. In addition, sIL-2R had higher sensitivity but lower specificity than ACE. Both PPV and NPV values favoured sIL-2R (cutoff ≥ 4000 pg/mL) over ACE (cutoff ≥51 U/L).

The sensitivity of sIL-2R reported herin for the diagnosis of sarcoidosis in patients with uveitis was lower than that reported by Gundlach *et al*[5] (81% vs 98%).This discrepancy might be explained by the lower cutoff level for sIL-2R used in that study. Gundlach *et al* also reported higher sIL-2R specificity (94% vs our finding of 64%), which might be explained by their inclusion of patients (20 of 42; 48%) with probable and possible sarcoidosis, diagnoses that are based solely on laboratory and clinical signs.[2,5] The inclusion of patients with only presumed and definitive ocular sarcoidosis in the present study, which was based on histologic and radiologic criteria, enabled an unbiased evaluation of sIL-2R and ACE levels, giving a lower proportion of true-negatives and thus lower specificity.[2]

Our results showed that high sIL-2R levels also occurred in patients with uveitis that was not associated with sarcoidosis, indicating a high proportion of T-cell-mediated disease in the population with uveitis. Our study results highlighted the need for using different cutoffs for diagnostic tests in diverse populations. An sIL-2R level above the reference value of 2500 pg/mL indicates increased T-cell activity compared with that in a healthy population. However, an optimized cutoff should be determined for diagnostic purposes in disease populations.

We found low serum sIL-2R levels in patients with birdshot chorioretinopathy and with Vogt-Koyanagi-Harada syndrome, a finding that may help distinguish these ocular disorders from sarcoidosis. However, because the numbers of patients with birdshot chorioretinopathy and Vogt-Koyanagi-Harada syndrome were limited in our study, the low sIL-2R levels should be confirmed in larger studies.

The clinically most useful diagnostic test combination for sarcoidosis in patients with uveitis was the determination of serum sIL-2R levels combined with chest radiography (sensitivity and specificity of 92% and 58%, respectively). The high sensitivity of this combination reduces the chance of missing sarcoidosis compared with that afforded by the current clinical practice of determining serum ACE levels and obtaining a chest radiography (sensitivity of 70%).

Limitations

Our study has some shortcomings inherent in retrospective studies. Not all samples were obtained during active ocular disease, which might be associated with lower levels of sIL-2R and ACE.[12] In our study, no association was found between ocular disease activity and elevated serum sIL-2R or ACE levels. These serum measurements reflected overall disease activity, but disease activity limited to the eyes may not be accurately reflected by these serum factors.

Not all of the patients in the present study underwent chest radiography shortly after the onset of uveitis, which might have influenced the percentage of positive and negative chest radiographic findings and certainly influenced the elevated PPFV of chest radiography found in the present study. Levels of serum sIL-2R and ACE fluctuate over time with the activity of sarcoidosis and are not associated with changes in the same way as those observed over time on chest radiography.

We detected high variability in the serum sIL-2R levels that could not be explained by sex or age nor by the interassay variation coefficient. The high variability may reflect the systemic diseases represented in this cohort. Standard deviation can be influenced by the individual and mean values as well as by the sample size. The high variability in the individual values of patients included in the nonsarcoidosis-related groups likely increased the SD of the whole cohort.

Conclusion

This study indicates that the serum sIL-2R level is a useful biomarker for diagnosing sarcoidosis in patients with uveitis, showing an overall diagnostic performance slightly better than that of serum ACE levels.

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chapter 3.4

Lymphopaenia as a Predictor of Sarcoidosis in Patients with a First Episode of Uveitis

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Br J Ophthalmol. 2018 Nov 15; 0:1–5.

Abstract

Background/ Aims The diagnostic properties of conventional diagnostic tests (angiotensin converting enzyme and chest radiography) for sarcoidosis-associated uveitis are not ideal. The diagnostic value of lymphopaenia for sarcoidosis-associated uveitis is investigated.

Methods A retrospective study of 191 consecutive patients with a first uveitis episode visiting the ophthalmology department (Erasmus Medical Center, Rotterdam, The Netherlands). Receiver operating characteristics (ROC) analysis was performed and compared to known ROC values from literature of conventional diagnostic tests for sarcoidosis-associated uveitis. An ideal cut-off was determined for lymphopaenia by calculation of the highest Youden-index.

Results Out of all patients with first uveitis attack, 32/191;17% were subsequently diagnosed with biopsy-proven or radiological diagnosis of sarcoidosis. Lymphopaenia (<1.5 $x10^9$/L) was significantly more often observed in sarcoidosis-associated uveitis patients compared to non-sarcoidosis-associated uveitis patients ($P<0.05$). The sensitivity and specificity of lymphopaenia was 75% and 77%, respectively. The optimal cut-off for lymphopaenia for diagnosing sarcoidosis-associated uveitis was 1.47 $x10^9$/L. Lymphopaenia resulted in a 12.0 (95% confidence interval (CI); 4.7-30.5) fold risk for having sarcoidosis, corrected for sex, race and age at onset of uveitis in patients with a first uveitis attack.

Conclusion Lymphopaenia is a non-invasive and useful marker for diagnosing sarcoidosis associated uveitis.

Introduction

Ocular involvement is frequently observed in sarcoidosis, usually manifesting as uveitis.[1] Diagnosis of sarcoidosis-associated uveitis can be challenging, since ocular histology (the golden standard for the diagnosis of sarcoidosis) is difficult and uveitis may precede extraocular manifestations of sarcoidosis.[2] Non-invasive diagnostic tests for sarcoidosis are therefore attractive in patients presenting with uveitis. Chest X-ray and serum biomarkers (Angiotensin Converting Enzyme (ACE) and lysozyme) are regarded diagnostic and classifying tests according to the International Workshop On Ocular Sarcoidosis (IWOS) criteria.[3] However, these tests have limited predictive values (PPV).[3]

In sarcoidosis, T-lymphocytes are activated and skew from the peripheral blood to the affected tissue, resulting in a relative (T cell) lymphopaenia.[4-6] Soluble interleukin 2 receptor (sIL-2R) reflects activation of T-lymphocytes. The diagnostic value of sIL-2R and ACE in the diagnosis of sarcoidosis-associated uveitis seem similar in earlier investigations.[7] It has been suggested that the diagnostic criteria of sarcoidosis-associated uveitis may be modified by inclusion of lymphopaenia.[8]

Herein, we study the value of lymphopaenia as a diagnostic biomarker for sarcoidosis-associated uveitis in a therapy naive population with a first episode of uveitis.

Material And Methods

The “Strengthening the Reporting of Observational studies in Epidemiology” guidelines were used for reporting this observational study.[9] Retrospective use of laboratory investigations adheres to the tenets of the Declaration of Helsinki and the Erasmus Medical Center medical ethical committee approved the bio banking protocol and associated procedures.

Study Population

We performed a study of 191 patients with a new onset of uveitis visiting the ophthalmology department at the Erasmus University Medical Center, Rotterdam, the Netherlands from January 2011-July 2017. All patients presenting with a first episode of uveitis and available lymphocyte counts within one month after the onset of uveitis were included. Data were reviewed retrospectively between January and September 2017. Patients with a known cause of lymphopaenia were excluded (Supplementary table 1).

Originally, 244 patients with a first episode of uveitis were identified. 53 patients with a known cause for lymphopaenia were excluded (Immunosuppressive medication N= 17; Infectious disease N= 21; systemic disease N=5; immunosuppressive medication and systemic disease N=7; immunosuppressive medication and infection N = 3). The remaining 191 patients were included in the present study.

Definition of diagnostic categories

The etiologic cause of uveitis was determined after the initial diagnostic work-up in our centre. The diagnosis of sarcoidosis was based on the International Workshop on Ocular Sarcoidosis (IWOS) criteria (only definitive and presumed ocular sarcoidosis patients i.e. biopsy or radiological confirmations were categorized as sarcoidosis).[3] Controls were the remainder of (non-sarcoidosis) patients with first attack of uveitis.

Other diagnoses were established according to current international criteria.[10-16] A definite diagnosis of tuberculosis (TB)-associated uveitis was based on a positive microbiology test anywhere in the body without other explanation of uveitis.

Assessment of variables

Demographic data, the onset of uveitis, laterality, location of the uveitis, and the final diagnosis of uveitis were noted. The uveitis onset was defined as the date on which an ophthalmologist first documented uveitis.

A multiple linear regression was performed to identify a possible relationship between absolute lymphocyte counts and the duration of uveitis (the duration between moment of uveitis onset until the day of blood sampling). Corrected for sex and race, there was no association between the lymphocyte counts and uveitis duration (≤1 month, p=0.126). Therefore, only patients that had lymphocytes recorded within 1 month after or before the diagnosis of uveitis were included. For this study, the general cut-off for lymphopaenia was used (<1.5 $x10^9$/L).

Statistical analysis

The characteristics of patients were summarized using descriptive statistics, such as means and percentages. Non-parametric tests were used to compare characteristics between the groups.

The sensitivity and specificity of lymphopaenia as well as the C-statistic (the area under the receiver operating characteristic (ROC) curve; a measure of test performance) for the diagnosis of sarcoidosis was calculated. The ROC and Youden's index, (sensitivity + specifity -1), was used to summarize test performance.[17] The optimal cut-off for lymphopaenia in the diagnosis of sarcoidosis-associated uveitis was calculated by maximizing the the Youden's index.

Binary logistic regression was used to measure the significance of association between lymphopaenia and sarcoidosis-associated uveitis, corrected for gender, race, age at onset of uveitis, use of any immunosuppressive treatment, immunosuppressive disease or immunosuppressive infection. The statistical analysis was done using Excel, IBM SPSS statistics 21.0.0 for Windows (SPSS inc., Chicago, IL, USA), and R, using the package pROC.

Results

The characteristics of our study population are depicted in Table 1. The mean age of onset of uveitis was 46.8 ± 18.0 years and 120/191; 63% of patients were female and 128/191; 67% of Caucasian descent. The median interval between onset of uveitis and determination of lymphocyte count was 4.0 days. All patients had their first episode of uveitis without a known etiology of their uveitis at moment of blood sampling.

Diagnoses of uveitis were performed after all relevant tests were performed (Table 1). Sarcoidosis-associated uveitis was diagnosed in 32 patients (17%) and the mean onset of uveitis in this group was 45.5 years ±17.3 and showed slight preponderance of female gender (21/32; 66%) and Caucasian ancestry (19/32; 59%). These characteristics did not differ compared to the non-sarcoidosis-associated uveitis patients (N=159; P>0.05).

Panuveitis and bilateral involvement were more common in sarcoidosis-associated uveitis (24/32;75% and 26/32; 81%, respectively) compared to non-sarcoidosis-associated uveitis patients (70/159;44% and 76/159;48%), P=0.002 and P=0.001, respectively.

Optimal cut-off lymphocyte counts for the diagnosis of sarcoidosis-associated uveitis

The cut-off that corresponded with the highest Youden Index (0.54) was (1.47 x10^9/L) The associated sensitivity and specificity was 75% and 79%, respectively.

TABLE 1. Basic characteristics of consecutive patients with a first uveitis attack.

	Total (N=191)
Mean age (years) at onset of uveitis (mean ± SD)	46.8 (±18.0)
Median interval (days) between onset of uveitis and determination of lymphocyte count (range)	4 (-31,11)
Gender	
Males	71/191 (37%)
Females	120/191 (63%)
Race	
Caucasian	128/191 (67%)
Non-Caucasian	63/191 (33%)
Laterality	
Unilateral	89/191 (47%)
Bilateral	102/191 (53%)

TABLE 1. Continued.

	Total (N=191)
Anatomical localization of uveitis	
Anterior	35/191 (18%)
Intermediate	11/191 (6%)
Posterior	43/191 (23%)
Panuveitis	94/191 (49%)
Scleritis	8/191 (4%)
Associated with systemic disease	**65/191 (34%)**
Sarcoidosis	32/65 (49%)
Definitive sarcoidosis	*24/32 (75%)*
Presumed sarcoidosis	*8/32 (25%)*
HLA B27-associated uveitis	8/65 (12%)
VKH- syndrome	6/65 (9%)
Inflammatory bowel disease	4/65 (6%)
Behçet's disease	4/65 (6%)
Multiple sclerosis	4/65 (6%)
Miscellaneous[a]	7/65 (11%)
Infectious uveitis	**30/191 (16%)**
Toxoplasmosis	18/30 (60%)
Varicella-zoster-associated uveitis	3/30 (10%)
Herpes-simplex-associated uveitis	2/30 (7%)
Miscellaneous[b]	7/30 (23%)
Established clinical entity	**23/191 (12%)**
Masquerade syndrome[c]	9/23 (39%)
BSCR	4/23 (17%)
White dot syndrome	4/23 (17%)
Miscellaneous[d]	*6/23 (26%)*
Unknown	**73/191 (38%)**
QFT-	42/73 (58%)
QFT not performed	24/73 (33%)
QFT+	7/73 (10%)

SD = standard deviation, IQR = interquartile range, HLA = human leukocyte antigen, VKH = Vogt-Koyanagi- Harada, BSCR = birdshot chorioretinopathy, AMPPE = Acute Multifocal Placoid Pigment Epitheliopathy, IGRA = Interferon Gamma Release Assay.

[a]Including granulomatosis with polyangiitis (N=2), reactive arthritis associated with uveitis (N=1), Kikuchi's disease (N=1), juvenile idiopathic arthritis (N=1), Acute Disseminated Encephalomyelitis (N=1), Devic's disease (N=1).

[b]Including endogenous endophthalmitis (N=2), tuberculosis (N=2), rubella-virus-associated uveitis (N=1), bartonella henselae (N=1), borrelia burgdorferi (N=1).

[c]Including lymphoma (N=3), macular dystrophy (N=2), drusen (N=1), schwannoma (N=1), central serous chorioretinopathy (N=1), coats disease (N=1).

[d]Including toxic uveitis (N=3), post-operative uveitis (N=2), sympathetic ophthalmia (N=1).

Lymphopaenia

Lymphopaenia was present in 61 out of 191 patients (32%) out of which 24/61 (39%) had sarcoidosis (21 with biopsy proven and 3 with presumed sarcoidosis). The remaining patients with lymphopaenia included HLA-B27-associated uveitis, Behçet's disease, multiple sclerosis, granulomatosis with polyangiitis and Vogt-Koyanagi-Harada syndrome (Table 2). Lymphopaenia in infectious uveitis was seen in 5/61; 8% (including endogenous endolphthalmitis, active tuberculosis, toxoplasmosis and herpes-simplex-associated uveitis). The proportion of lymphopaenia in patients with established cause of uveitis and uveitis of unknown cause did not differ, P=0.52.

Sarcoidosis-associated uveitis

Lymphopaenia was significantly (P=0.0001) more observed in sarcoidosis-associated uveitis patients than in non- sarcoidosis-associated uveitis patients (24/32; 75% vs. 37/159; 23%, respectively). Furthermore, the mean lymphocyte count in sarcoidosis-associated uveitis patients was significantly lower than in non-sarcoidosis patients (1.3 ± 0.5x10^9/L and 2.0 ± 0.8x10^9/L; P=0.0001, respectively).

Sarcoidosis-associated uveitis and lymphopaenia

Sarcoidosis-associated uveitis patients with lymphopaenia (N=24) were mostly female (14/24;58%) of Caucasian origin (14/24; 58%) with panuveitis (18/24;75%) and bilateral involvement (19/24; 79%). Sarcoidosis-associated uveitis patients with and without lymphopaenia were similar in location of uveitis and prevalence of bilateral involvement (panuveitis in 18/24;75% vs. 6/8; 75%, P=1.0 and 19/24;79 vs. 7/8;88, P=1.0, respectively).

Corrected for sex, race and age at onset of uveitis, the occurrence of lymphopaenia increased the risk to find sarcoidosis with a factor 12.0 (95% confidence interval (CI); 4.7-30.5) fold risk for having sarcoidosis, corrected for sex, race and age at onset of uveitis (Table 3).

Test characteristics of lymphopaenia in sarcoidosis-associated uveitis

Table 4 depicts the various test characteristics of lymphopaenia. has a sensitivity of 75% (95% CI; 60.0-90.0) and a specificity of 76% (95% CI; 70.2-83.3). This corresponds to a Youden's index of 0.517 and C-statistic of 0.792 (0.710-0.874). Table 5 summarizes commonly used diagnostic tests for the detection of sarcoidosis-associated uveitis, including previous literature.

Sensitivity analysis

A sensitivity analysis was performed, adding patients with known causes of lymphopaenia in their history (in total N=244). When analyzing this group the PPV became lower (28%) and the sensitivity and specificity were different (72% and 68%, respectively) when compared to our population without obvious causes for lymphopaenia (N = 191).

TABLE 2. Lymphocyte counts in different etiologic categories of patients with a first uveitis attack.

	Total	**Lymphocyte count <1.5x10⁹ /L /L**	**Lymphocyte count ≥1.5x10⁹**
	(N=191)	**(N=61)**	**(N=130)**
Associated with systemic disease	**65/191 (34%)**	**32/65 (49%)**	**33/65 (51%)**
Sarcoidosis	32/65 (49%)	24/32 (75%)	8/32 (25%)
Definitive sarcoidosis	*24/32 (75%)*	*21/24 (87%)*	*3/24 (13%)*
Presumed sarcoidosis	*8/32 (25%)*	*3*/8 (38%)	*5/8 (63%)*
HLA B27-associated uveitis	8/65 (12%)	2/8 (25%)	6/8 (75%)
VKH- syndrome	6/65 (9%)	1/6 (17%)	5/6 (83%)
Inflammatory bowel disease	4/65 (6%)	1/4 (25%)	3/4 (75%)
Behçet's disease	4/65 (6%)	2/4 (50%)	2/4 (50%)
Multiple sclerosis	4/65 (6%)	1/4 (25%)	3/4 (75%)
Miscellaneous	7/65 (11%)	1/7 (14%)[a]	6/7 (86%)
Infectious uveitis	**30/191 (16%)**	**5/30 (17%)**	**25/30 (83%)**
Toxoplasmosis	18/30 (60%)	1/18 (6%)	17/18 (94%)
Varicella-zoster-associated uveitis	3/30 (10%)	0	3/3 (100%)
Herpes-simplex-associated uveitis	2/30 (7%)	1/2(50%)	1/2(50%)
Miscellaneous	7/30 (23%)	3/7 (43%)[b]	4/7 (57%)
Established clinical entity	**23/191 (12%)**	**3/23 (13%)**	**20/23 (87%)**
Masquerade syndrome	9/23 (39%)	2/9 (22%)[c]	7/9 (78%)
BSCR	4/23 (17%)	0	4/4 (100%)
White dot syndrome	4/23 (17%)	0	4/4 (100%)
Miscellaneous	*6/23 (26%)*	1/6 (17%)[d]	5/6 (83%)
Unknown	**73/191 (38%)**	**21/73 (29%)**	**52/73 (71%)**
QFT-	42/73 (58%)	11/42 (26%)	31/42 (74%)
QFT not performed	24/73 (33%)	8/24 (33%)	16/24 (67%)
QFT+	7/73 (10%)	2/7 (29%)	5/7 (71%)

SD = standard deviation, IQR = interquartile range, HLA = human leukocyte antigen, VKH = Vogt-Koyanagi- Harada, BSCR = birdshot chorioretinopathy, AMPPE = Acute Multifocal Placoid Pigment Epitheliopathy, IGRA = Interferon Gamma Release Assay.

[a]Including granulomatosis with polyangiitis (N=1).

[b]Including endogenous endolphthalmitis (N=2), active tuberculosis (N=1).

[c] Including non-Hodgkin lymphoma (N=1) and Hodgkin lymphoma (N=1).

[d] Including toxic anterior uveitis syndrome (N=1).

TABLE 3. Odds ratios of lymphopaenia corrected for possible confounders in patients with a first uveitis attack.

	Sarcoidosis OR (95%CI)
Lymphocytopenia (<1.5x10^9/L)	12.0 (4.7-30.5)
Sex	1.8 (0.7-4.6)
Race	0.9 (0.4-2.4)
Age at onset uveitis	1.0 (1.0-1.0)

OR = odds ratio, CI = confidence interval.
Dependent variable = diagnosis of sarcoidosis-associated uveitis. Independent variables: lymphocyte count (with different cut-offs), gender, race (Caucasian, non-Caucasian), age at onset of uveitis (years), immunosuppression (either therapy, any immunosuppressive systemic disease or infection).

TABLE 4. Diagnostic properties of lymphopaenia in the diagnosis of sarcoidosis in patients with a first uveitis attack.

	Lymphopaenia <1.5x10^9/L OR (95%CI)
Sensitivity (95% CI)	75% (60.0-90.0)
Specificity (95% CI)	76% (70.2-83.3)
Youden's index	0.517
NPV	0.938
PPV	0.393
C-statistic	0.792 (0.710-0.874)

OR = Odds Ratio, CI = Confidence Interval, NPV = negative predictive value, PPV = positive predictive value

Discussion

This retrospective study demonstrates that lymphopaenia was strongly associated with the diagnosis of sarcoidosis in patients with a first episode of uveitis. The cut-off for lymphopaenia with most ideal test characteristics was 1.47 $x10^9$ L, close to the general cut-off used in this study (1.5 $x10^9$ L), which also might be used.

Peripheral T-lymphocytes are decreased in sarcoidosis and may be an appropriate screening tool in uveitis patients. [6,8,18] Therefore, lymphocyte counts have recently been proposed by Jones et al to be added to the diagnostic IWOS criteria for sarcoidosis-associated uveitis (with cut-off <1.0x10^9/L and corresponding OR of 5.7).[8] The lymphocyte values found in Jones' study however, cannot be implemented in patients with a new onset of uveitis, because patients with a second or further episode of uveitis were also included. Furthermore, not all patients have been diagnosed according to the IWOS criteria (patients with elevated serum markers, but without biopsy or radiological confirmations were also labeled as sarcoidosis-associated uveitis). The optimal cut-off, identified in this study (1.47x10^9/L), is close to the general cut-off for lymphopaenia used in this study (1.5 $x10^9$/L) but differs from the proposed cut-off by Jones *et a*l (<1. 0x10^9/L). Since the optimal cut-off is very similar to the general cut-off for lymphopaenia, the general cut-off can be used to diagnose sarcoidosis (with comparable test characteristics).

Predictive values indicate the chance of disease in a patient with a positive test result (PPV) or the chance that the patient does not have the disease when the test is negative (NPV). Since conventional diagnostic tests have low PPV values in diagnosing sarcoidosis-associated uveitis, a search for a more sensitive and specific diagnostic test is warranted.[7,8] Lymphopaenia has a higher PPV than for ACE and soluble interleukine-2 receptor (sIL-2R), but lower when compared to chest X-ray (Table 5).[7,19] The NPV (ruling out sarcoidosis when a test is negative) of lymphopaenia is comparable to that of chest X-ray and higher than the NPV of ACE in previous studies (Table 5). Absence of lymphopaenia therefore performs better than normal ACE levels in ruling out sarcoidosis in the uveitis population and its performance is comparable to chest X-ray, but is less invasive and less expensive compared to the latter. The diagnostic value of the combinations of various tests including lymphopaenia, sIL-2R and chest X-ray would be interesting to investigate, since this is scarcely touched upon in the current literature, but are beyond the scope of this study.

Other predictive factors for sarcoidosis in our uveitis population were panuveitis and bilateral involvement. Preponderance of female gender in ocular sarcoidosis has already been described, an aspect we did not identify in our population as a risk factor. [1,20,21] Our study did not contain many Asian patients, a known predictive factor for ocular involvement in

sarcoidosis.[22-27] Therefore in this study, this association could not be established. Panuveitis and bilateral involvement were more common in sarcoidosis-associated uveitis compared to non-sarcoidosis patients. Future research should elaborate on the value of combining the epidemiologic features together with laboratory tests and imaging in differentiating sarcoidosis-associated uveitis from other causes of uveitis. Since the number of patients was limited, the detailed assessment of sarcoidosis-associated uveitis for patients with and without lymphopaenia was not performed.

TABLE 5. Summary of test characteristics of lymphopaenia, angiotensin converting enzyme, chest X-ray and soluble interleukine 2 –Receptor levels for the diagnosis of sarcoidosis-associated uveitis based on previous literature and the present study.

	Sensitivity	Specificity	PPV	NPV	AUC
Lymphopaenia *(<1.5x10^9/L)*					
Present study	75%	77%	0.39	0.94	0.79
Chest X-ray					
Groen et al, 2017	64%	91%	0.47	0.95	NA
sIL-2R *(<4000 pg/mL)*					
Groen-Hakan and Eurelings et al, 2017	81%	64%	0.28	0.95	0.76
ACE *(≥ 68 U/mL)*					
Groen-Hakan and Eurelings et al, 2017	30%	85%	0.26	0.87	0.65

PPV = positive predictive value, NPV = negative predictive value, AUC = area under the curve, ACE = Angiotensin Converting Enzyme, NA = not applicable, sIL-2R = soluble interleukine 2- Receptor.

The differential diagnosis of uveitis is diverse: from infectious etiologies to auto-inflammatory/immune diseases.[28,29] In the present study most patients with lymphopaenia have either sarcoidosis or idiopathic uveitis (45/61;74%). However, the association of lymphopaenia with other etiologic groups cannot be entirely excluded, due to the limited number of patients in specific diagnostic categories of uveitis and might geographically vary. The predictive value of lymphopaenia depends on the prevalence of sarcoidosis in the uveitis population. The use of lymphocytes as a predictor of sarcoidosis associated uveitis might therefore be limited in settings, where other diseases are more prevalent, such as Tuberculosis endemic countries. Our University Center is a sarcoidosis center for the region of South-Holland, thus probably containing a higher proportion of sarcoidosis patients when compared to other University Centers.

There are multiple causes for lymphopaenia, such as receiving immunosuppressive medication or presence of diseases that dysregulate the immune system (such as HIV). Patients with a known cause of lymphopaenia have been excluded from this study (inferior test characteristics were observed in our sensitivity analysis including patient with known cause of lymphopaenia). Therefore, in patients with a known reason for lymphopaenia, a determination of a lymphocyte count is probably not helpful for screening purposes.

Confirmation bias, which is introduced if the outcome (sarcoidosis) precedes the assessment of the variable (lymphocyte count) was minimized in our study since the lymphocyte counts were always measured before the diagnosis was made.

In conclusion, lymphopaenia appears an useful diagnostic biomarker for the diagnosis of sarcoidosis in patients experiencing their first uveitis attack. Further avenues of research should concentrate on the development of other noninvasive tests for the diagnosis of ocular sarcoidosis and selecting the optimal combination of available tests.

Supplementary Data

Supplemental Table 1 Causes of lymphopaenia
http://dx.doi.org/10.1136/bjophthalmol-2018-313212

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chapter 4

Prevalence of positive QuantiFERON-TB gold in-tube test in uveitis and its clinical implications in a country non-endemic for tuberculosis

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Submitted for publication.

chapter 5

Viral (anterior) Uveitis and its Diagnostic Tests

chapter 5.1

Challenges of Diagnosing Viral Anterior Uveitis

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Ocul Immunol Inflamm. 2017 Oct;25(5):710-720.

Abstract

The viral causes of anterior uveitis (AU) emerged with the use of novel molecular diagnostic tests and serologic tests adapted for small volumes (Goldmann-Witmer Coefficient). The viral causes of AU may be underestimated, and some of the presumed idiopathic AU cases will probably be proven to be of viral origin in the coming years. So far, a viral origin of AU was suspected in patients who presented with unilateral hypertensive AU. It is not clear which clinical presentations should raise a suspicion of viral etiology. There is an overlap in the clinical manifestations of AU caused by viruses and other non-viral forms of AU. A viral cause of AU should be suspected in patients with unilateral AU, exhibiting small or medium sized KPs, some form of iris atrophy, high IOP and early development of a cataract and the definitive diagnosis can be proven by aqueous humor analysis.

Introduction

Anterior uveitis (AU) is the most common anatomic type of uveitis encountered by ophthalmologists.[1] Though traditionally reported that most cases of AU are of unknown origin, the specific etiology may presently be documented in a substantial number of cases. The AU has multiple causes and in adults, the most frequent entity is Human Leukocyte Antigen-B27 (HLA-B27)-associated uveitis, whilst juvenile idiopathic arthritis (JIA)-associated AU is the most frequent entity occurring in children.[1, 2] The viral causes of AU emerged with the use of novel molecular diagnostic tests and serologic tests adapted for small volumes (Goldmann-Witmer Coefficient; GWC). The viral causes of AU may be underestimated, and some of the presumed idiopathic AU cases will probably be proven to be of viral origin in the coming years.

The most common AU-inciting infections and associated systemic diseases are given in Table 1. Herpes simplex virus (HSV) and varicella zoster virus (VZV) represent common viral causes of AU in the West, whilst cytomegalovirus (CMV) is more frequent in Asia.[1, 3, 4] In contrast to decreasing prevalence of rubella virus (RV)-associated AU in vaccinated populations, novel uveitis entities such as Ebola virus and Zika virus-associated uveitis were discovered during recent epidemics.[5]

So far, a viral origin of AU was suspected in patients who presented with unilateral hypertensive AU. Further, distinctive signs were described for separate viruses, but it is not clear which clinical presentations should raise a suspicion of viral etiology. Herein we summarize the typical clinical manifestations of the common types of AU encountered in clinical practice and attempt to delineate the clinical characteristics commonly seen in patients with viral AU.

Clinical Features of Common Non-Infectious Anterior Uveitis Entities

Human Leukocyte Antigen-b27-associated anterior uveitis

Human leukocyte antigen-B27-associated uveitis is characterized by unilateral alternating acute non-granulomatous AU with marked fibrinous reaction or hypopyon (Figure 1), occurring typically in young adults, and has a frequent association with seronegative arthritic syndromes, of which the most prevalent is ankylosing spondylitis. Patients typically present with sudden onset of a classic triad of pain, redness and photophobia. The main external signs are conjunctival and perilimbal redness. The anterior segment shows diffuse cells and flare in the anterior chamber; sometimes with cells adhering to corneal endothelium, but large keratic precipitates (KPs) are not present. Intraocular pressure (IOP) often decreases in the acutely inflamed eye, but in severe cases, a fibrinous exudate and posterior synechiae may occlude the entire pupil leading to iris bombé and dramatic elevations in IOP. Less typical presentations involve posterior segment involvement including vitritis with or without pars plana exudates, optic disc swelling or papillitis, and cystoid macular edema (CME). Chronic AU as well as episcleritis and scleritis are less typical.[2]

Sarcoidosis-Associated Anterior Uveitis

Sarcoidosis may be associated with all anatomical types of uveitis. Anterior uveitis due to sarcoidosis is typically seen in young adults, more often in non-Caucasian races. The patient with sarcoidosis-associated AU may present with a few complaints and a relatively white eye. Raised IOP is often noted. Sarcoidosis-associated AU shows predominantly bilateral granulomatous inflammation with large fatty KPs located in the inferior part of the cornea (Arlt's triangle, Figure 2) and has sometimes also characteristic granulomatous lesions on the iris such as Koeppe and/or Busacca nodules. Posterior and anterior synechiae are common in this entity, and may be associated with the development of glaucoma.[6] Cystoid macular edema is a common complication of sarcoidosis-associated AU.[7,8] The presence of systemic sarcoidosis in a patient with uveitis of unknown origin is generally accepted as a confirmation of sarcoidosis-associated AU. Ocular signs suggestive of sarcoidosis were defined by the International Workshop on Ocular Sarcoidosis (IWOS).[6]

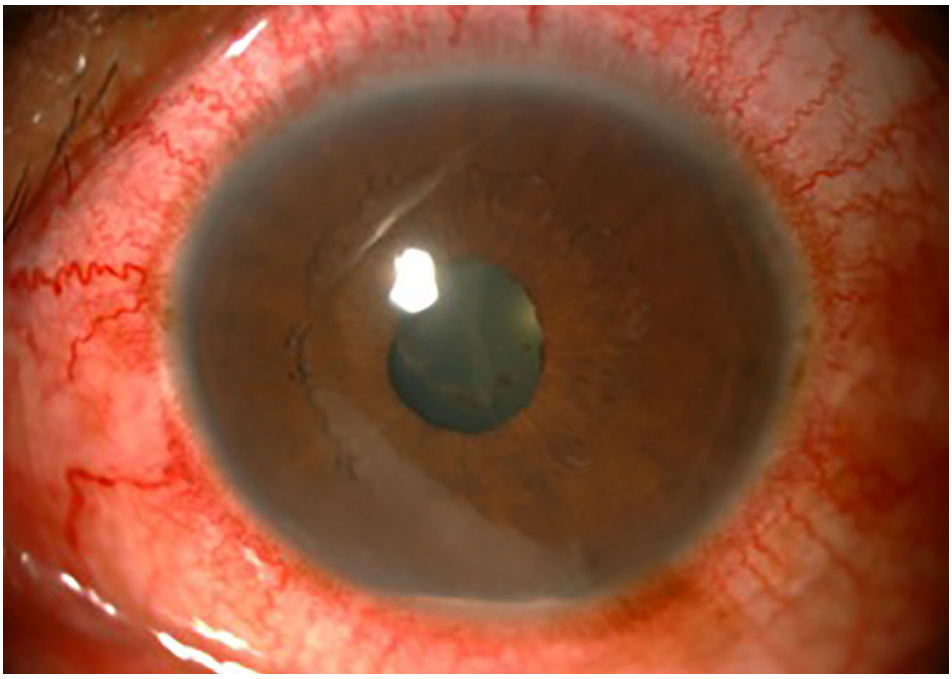

FIGURE 1. Conjunctival and perilimbal redness with fibrinous reaction seen in the pupil and hypopyon in patient with HLA B27-associated AU.

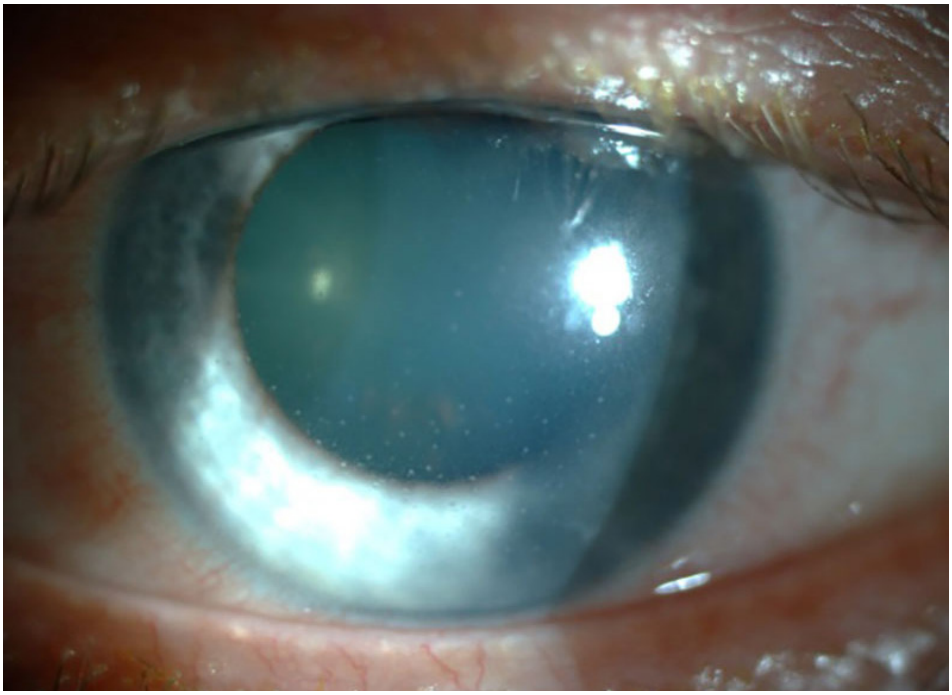

FIGURE 2. Keratic precipitates in sarcoidosis-associated anterior uveitis.

Juvenile Idiopathic Arthritis-Associated Anterior Uveitis

Anterior uveitis is the most common anatomic localization found in childhood uveitis and is associated with JIA in approximately 80% of the cases.[9] Development of uveitis is most common among patients with oligo-articular, rheumatoid factor-negative and psoriatic arthritis subtypes. Antinuclear antibodies (ANAs) are positive in 90% of the patients.[10] Clinical features of JIA-associated uveitis include mostly bilateral non-granulomatous inflammation, anterior in location, insidious at onset with chronic course. Juvenile idiopathic arthritis-associated AU is frequently initially asymptomatic. Uveitis in JIA can worsen over time as a result of many sight-threatening complications, such as band keratopathy in the visual axis, posterior synechiae, cataract, secondary glaucoma, macular edema, hypotony, epiretinal membrane and optic nerve edema. Different studies have pointed out that several factors are associated with poor prognosis, including young age at onset, male gender, short interval between diagnosis of arthritis and uveitis, severity of uveitis at onset and ANA positivity.[11-14] Unilateral permanent visual loss at the age of 18 was observed in 33% of the patients and bilateral visual loss occurred in 4%.[15] Although uveitis in JIA is considered a disease of childhood, the majority of the patients experience persistent ocular inflammation into adulthood.[15]

Tubulointerstitial Nephritis and Uveitis Syndrome

Tubulointerstitial nephritis and uveitis (TINU) syndrome affects mostly young patients with a peak incidence at the age of 14 years.[16] It accounts for 1-2% of all uveitis patients in specialized centers, but this number is probably underestimated since the nephritis component is often self-limiting and therefore not recognized.[17] Uveitis in TINU syndrome has mostly a chronic bilateral course and is frequently classified as AU in the literature, however vitritis may be prominent.[18, 19] Definitive diagnosis of TINU syndrome is based on histopathological examination of renal tissue. However, renal biopsy is not being performed in mild cases, because of the associated risks.[16] Probable TINU syndrome can be diagnosed by abnormal renal function urine analysis and systemic illness in the presence of uveitis.[16] The combination of urinary β-microglobulin and serum creatinine is a relatively simple screening tool for renal dysfunction in order to diagnose probable TINU syndrome in young patients with uveitis.[18]

Toxic uveitis

Past and current medication history may reveal an association of AU with the development of inflammatory or toxic reactions to diverse medications used by various routes.[20-22] Topical prostaglandin analogues may cause an acute non-granulomatous or chronic granulomatous AU. Topical brimonidine has been associated with a chronic AU, characterized by diffusely distributed stellate or micro-granulomatous KPs and a mild anterior chamber reaction, with or without concurrent conjunctivitis.[23] Intravitreal triamcinolone acetonide or anti-vascular endothelial growth factor (VEGF) injections may cause a mild AU or a sterile endophthalmitis with hypopyon in more severe cases.[20] An acute bilateral hypopyon may develop in

immunocompromised patients who receive rifabutin as prophylaxis against Mycobacterium avium complex.[20, 21] Biphosphonates that are used for the treatment of osteoporosis may cause an acute bilateral nongranulomatous AU with or without scleritis.[20-22] Intravenous or intravitreal administration of cidofovir, an antiviral agent used for the treatment of CMV retinitis, may cause non-granulomatous AU typically associated with ocular hypotony.[20, 21] Bacillus Calmette-Guérin (BCG) vaccination or intravesical BCG for the treatment of bladder cancer may rarely cause an acute bilateral non-granulomatous or granulomatous AU.[20] Melanoma or metastatic cancer patients receiving immune checkpoint inhibitors such as ipilimumab, pembrolizumab, or nivolumab may present with red eyes and mild or severe AU with posterior synechiae which may also be associated with keratitis.[22] Anti-tumor necrosis factor (TNF) agents, particularly etanercept, may cause a paradoxical intraocular inflammation, which may sometimes present as a sarcoid-like granulomatous anterior uveitis.[20, 21] Bilateral acute iris transillumination (BAIT) syndrome, which can mimic acute iridocyclitis, has been linked to oral fluoroquinolones, especially moxifloxacin (Figure 3). It is characterized by severe photophobia associated with bilateral pigment dispersion into the anterior chamber, diffuse iris trans illumination, and atonic distorted pupils.[24]

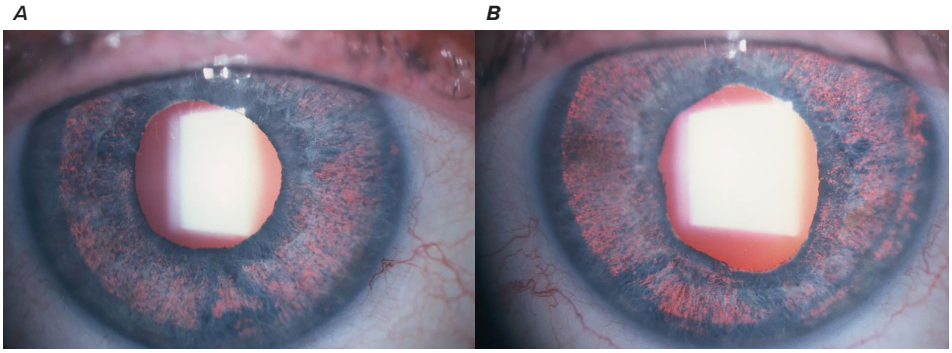

FIGURE 3. Diffuse iris transillumination and mild dilated distorted pupils in the right (A) and left (B) eye of a 56 year-old woman who had symptoms of bilateral acute iridocyclitis one month after the use of oral moxifloxacin for the treatment of urinary system infection.

Clinical Features of Common Bacterial Anterior Uveitis Entities

Syphilis-Associated Anterior Uveitis

Non-granulomatous as well as granulomatous inflammation with iris nodules and posterior synechiae may be the initial presentations of syphilis. Roseolae located on the iris represent a known feature in syphilis. Episcleritis, scleritis, keratitis and hypopyon were also reported. Increase in IOP can occur during active inflammation. The diagnosis is usually based on serological tests. The Centers for Disease Control and Prevention currently recommends Enzyme Immunosassays (EIAs) and Chemiluminescent Immunoassays (CIAs) to detect antibodies to treponemal antigens as the best screening tests for syphilis followed by testing of positive specimens with the non-treponemal test, rapid plasma regain (RPR). Specimens positive by EIA and CIA and negative on RPR are submitted for a confirmatory *Treponema pallidum* particle agglutination test and if positive, the diagnosis of syphilis is confirmed.[25, 26]

Tuberculosis-Associated Anterior Uveitis

Intraocular inflammation secondary to tuberculosis (TB) is common in developing countries. Patients with AU due to TB present with unilateral or bilateral symptoms of redness, pain, photophobia and floaters. Tuberculosis-associated AU can be markedly asymmetric. Adjacent ocular involvement in the form of scleritis, interstitial keratitis (Figure 4), phlycten and chronic conjunctivitis may also be seen. Anterior uveitis is characterized by medium to large KPs (Figure 5), which can be few or diffuse over the corneal endothelium. Pigmented hypopyon has also been reported in intraocular TB. Fibrin in anterior chamber may be seen in aggressive inflammation. Inflammation may also be accompanied by Koeppe or Busacca nodules, or by nodules located in the iridocorneal angle (Figure 5), which may lead to secondary glaucoma. Broad-based posterior synechiae may also be seen (Figure 5). Long standing chronic anterior uveitis may be associated with formation of pupillary membranes and iris neovascularisation. Anterior uveitis may be accompanied by posterior segment involvement like choroiditis, retinal vasculitis, choroidal tuberculomas, optic nerve granulomas and intermediate uveitis. Cataract and glaucoma are known complications seen in chronic AU. Confirmation of ocular TB is usually based on indirect evidence (diagnosis of systemic TB and/or good therapeutic response to anti-tuberculous therapy) and tests based on direct examinations of ocular tissues are less common. Molecular techniques performed on the intraocular fluids are becoming more widespread, but their clinical relevance is not yet clearly established).[27, 28]

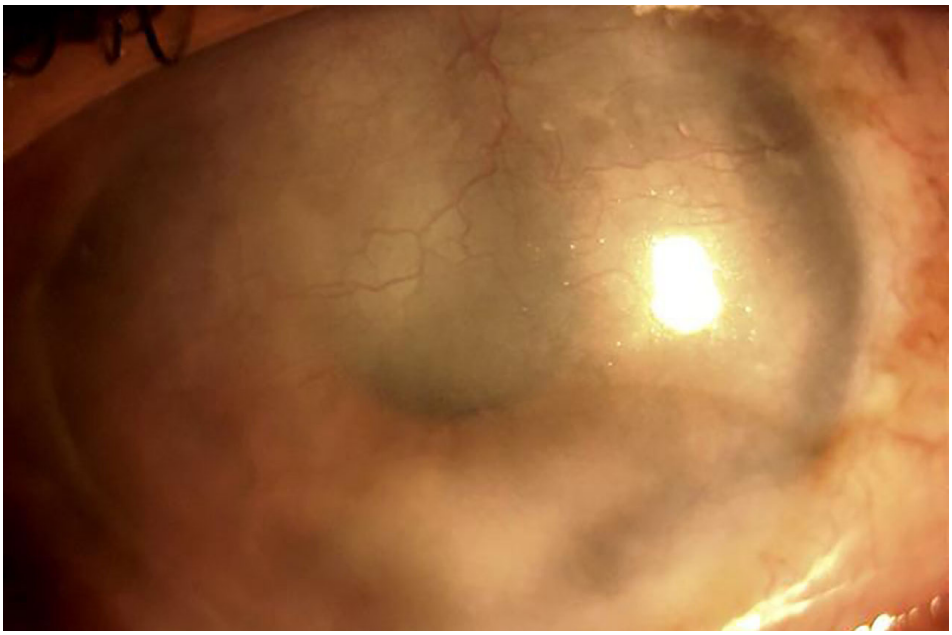

FIGURE 4. Interstitial keratitis due to tuberculosis.

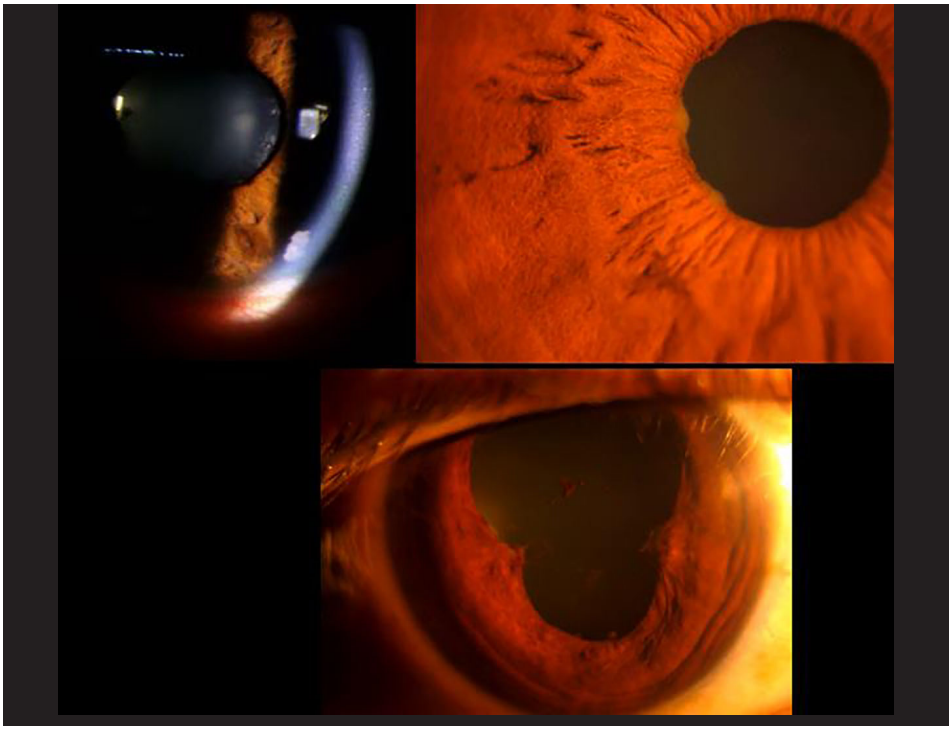

FIGURE 5. Large keratic precipitates with Koeppe nodules on the pupillary margin and broad posterior synechiae in ocular tuberculosis.

Clinical Features of Common Viral Anterior Uveitis Entities

Herpes Simplex Virus and Varicella Zoster Virus-Associated Anterior Uveitis

Herpes simplex virus and VZV, just as CMV belong to the Herpesviridae family. Following primary infection, life-long latency is a characteristic feature of this virus family.[29, 30] These viruses may present with AU, keratitis, dermatitis and/ or conjunctivitis.[29]

Common features of AU due to HSV or VZV infection are the unilateral localization and acute course commonly associated with subsequent recurrences or development of chronicity. Anterior chamber inflammation may be severe, and KPs of diverse types and sizes have been reported. An irregular pupil is a typical finding and is caused by iris atrophy (typically sectoral in HSV or more diffuse in VZV), which is caused by ischemic necrosis of iris stroma.[31] Intraocular pressure is usually elevated during the acute stage and subsequent development of glaucoma is common.

Associated corneal opacities in herpetic AU are commonly observed, but corneal involvement may be entirely absent. Herpes simplex virus-associated keratitis typically shows stromal inflammation with associated endotheliitis and fine corneal dendrites without elevated appearance, while VZV-associated keratitis is interstitial with corneal ring infiltrates and rough dendrites lacking terminal bulbs that can have an elevated appearance.[29, 30] Furthermore, patients with HSV or VZV-associated AU show decreased corneal sensation.[30, 32, 33] In contrast to HSV, VZV may show involvement of the vitreous.[34] HSV usually affects children and young adults, VZV is more often seen in elderly and immunocompromised patients. Primary infection with HSV is characterized by typical skin or mucosa lesions. Varicella zoster virus gives a skin rash with associated vesicles, preceded by pain, in the ipsilateral dermatome. In cases associated with uveitis, typically the tip of the nose is also affected (Hutchinson sign).

Cytomegalovirus-Associated Anterior Uveitis

Cytomegalovirus-associated anterior segment inflammation in non-HIV-infected patients has a spectrum of clinical presentations, including Posner-Schlossmann and Fuchs uveitis syndromes (FUS). Cytomegalovirus-associated AU may also present as corneal endotheliitis, with corneal edema ranging from a small localized area to diffuse bullous keratopathy, associated with mild AU. The IOP is often acutely or chronically elevated. Keratic precipitates may be non-granulomatous, granulomatous or stellate, and are usually located in the inferior half of the cornea. They may be diffuse, linear or show a ring pattern or may appear as a coin-like lesions. White, medium-sized, nodular lesions surrounded by a translucent halo are also possible. Vitritis or retinitis in these eyes is rare.[35, 36]

Rubella Virus-Associated Anterior Uveitis

Rubella virus (RV)-associated AU was reported as one of the causes of FUS.[5, 37, 38] However, RV-associated AU does not always fulfill the criteria of FUS. Patients with RV-associated AU are usually young adults at time of first ophthalmological presentation, and at that time typically have mild uveitis without synechiae, but may already have a cataract causing visual impairment. Unilateral involvement, the presence of fine KPs and diffuse iris atrophy are typical for RV-associated AU.[39] Multiple iris nodules, easily visible in brown eyes, might be overlooked in patients with a light iris. The absence of redness and pain are typical. Focal "toxoplasmosis-like" chorioretinal scars may be seen.[34] The presence of vitritis is frequent, and RV-associated AU may be mistaken for idiopathic intermediate uveitis.

Human T-Cell Lymphotropic Virus Type 1-Associated Anterior Uveitis

Southern Japan and Africa are the endemic areas for human T-cell lymphotropic virus type 1 (HTLV-1) infection. Major ocular symptoms of HTLV-1-associated uveitis are sudden onset of floaters and blurred vision, but pain, itching and foreign body sensation may also be reported.[40-42] On examination, typically uni- or bilateral mild iritis is seen, frequently associated with vitritis. Retinal vasculitis may also be seen.[40-42] Graves' disease is probably a risk factor for HTLV-1 associated uveitis and HTLV-1 associated uveitis appears to be related to HTLV-1 induced myelopathy, however these relationships require further investigation.[42, 43] In short, HTLV-1 associated uveitis is most frequently of intermediate type.

Human Immunodeficiency Virus (HIV)-Associated Anterior Uveitis

Human immunodeficiency virus (HIV) causes a multisystem disease that may also involve the eyes. The presence of intraocular HIV-1 RNA was shown in about one third of HIV-positive patients with infectious uveitis, but the HIV loads in the eye were typically lower than in plasma.[44] Human immunodeficiency virus-induced uveitis was reported in patients in whom HIV loads in intraocular fluids exceeded the plasma loads; these patients are typically highly active, anti-retroviral therapy (HAART)-naïve and have low CD4 counts.[45, 46] Patients with HIV-associated uveitis complain of decreased vision but pain or conjunctival hyperemia are characteristically absent. The anatomic location of uveitis is typically anterior associated with vitritis and resembles FUS, but is more frequently bilateral. There are no associated retinal lesions or scars, no findings suggestive of opportunistic infections, and patients do not respond to topical corticosteroid therapy. Anterior segment inflammation is mild; KPs are small and/or medium sized, and scattered on the whole corneal endothelium. After the administration of HAART, the intraocular inflammation disappears quickly, as the intraocular and plasma HIV loads decrease. Therefore, HIV-induced uveitis should be suspected in non-treated HIV-positive patients or in those in whom such treatment has failed or in HIV positive patients who have AU without any retinal lesions, no proven infectious cause and exhibit no response to topical corticosteroids.[47]

Chikungunya Virus-Associated Anterior Uveitis

Non-granulomatous AU may occur after a recent history of systemic chikungunya virus infection. Fine to medium sized KPs with pigmentation may be seen distributed all over the endothelium. The IOP may be increased at the time of active inflammation. A FUS pattern may also be seen in chikungunya virus-related AU.[48] Accompanying posterior segment involvement in the form of retinitis is seen in many cases. Confirmation is by polymerase chain reaction (PCR) for chikungunya viral RNA. Treatment is usually with anti-inflammatory agents, like NSAIDS and topical corticosteroids.[48-50]

Zika Virus-Associated Anterior Uveitis

Zika virus disease is a mosquito-borne infection transmitted by the *Aedes aegypti* mosquito. There are also reports describing infection following sexual, perinatal and blood transfusions. The Zika virus infection was first reported in Uganda, clinically showing a similar presentation to Dengue virus.[51] The disease is mild in adults with acute infection, and includes anterior uveitis with non-purulent conjunctivitis. It has a benign prognosis and is treated with topical steroids. In congenital infections, microcephaly is commonly described, and ocular findings include anterior segment abnormalities such as iris coloboma and lens subluxation.[52, 53]

Ebola Virus-Associated Anterior Uveitis

Survivors of Ebola virus infection in convalescent phase suffer a slow and painful recovery with development of many complications. Around 20% of survivors of Ebola virus infection develop uveitis (after recovery of systemic disease), suggesting that the virus remains viable in the eye.[54] It remains unclear whether Ebola-associated AU is caused by cytopathic effect of the virus or represents an immune response, but one study reports on the detection of Ebola virus in aqueous humor of a patient with uveitis after the clearance of viremia.[55, 56] Anterior uveitis has been reported, which usually presents with KPs and posterior synechiae. Cataract and ocular hypertension may also occur in Ebola-associated AU.[54] Approximately 40% of eyes become blind according to the World Health Organization classification. There are no known demographic and physical risk factors for development of uveitis in Ebola virus infection survivors, with the exception of higher viral blood load. Interestingly, optic neuropathy without uveitis was also reported.[57-60]

Uncertain Viral Anterior Uveitis Entities

Epstein Barr Virus-associated anterior uveitis

Epstein-Barr-virus (EBV) is also a member of the herpes virus family. It has repeatedly been reported as a cause of diverse types of uveitis, but the role of EBV in uveitis is not entirely clear, since PCR in aqueous fluid can be positive in EBV-infected patients without uveitis.[61-64] Evidence of intraocular EBV antibody synthesis in AU is scarce.[65, 66] However, antiviral treatment with valgancyclovir of presumed EBV uveitis has been reported to be beneficial

in patients presenting with uveitis and positive EBV serology. Epstein-Barr-virus- associated AU can be preceded by a flu-like prodrome and manifest as severe AU with fibrinous exudate in the acute stage, associated also with hyperemia and edema of the optic disc.

Parvovirus-associated anterior uveitis

Acute parvovirus B19 infection causes erythema infectiosum or fifth disease in children, sometimes with polyarthritis. Interestingly, after acute infection, serum autoantibodies may be measured in these patients, such as ANAs and rheumatoid factor.[67] This similarity to JIA, in which patients may also have arthritis accompanied by ANA formation, raises the suspicion of a link between parvovirus B19 and JIA. Specific intraocular antibody production has been reported in patients with JIA-associated uveitis.[68] There is little evidence that parvovirus B19 is a direct cause of uveitis, however; in rare instances, parvovirus B19-associated uveitis has been reported.[69-71] Parvovirus B19 DNA was detected in aqueous humor of occasional patients with uveitis, but was also found in patients with cataract and serous retinal detachment.[70, 72]

Clinical Syndromes In Anterior Uveitis

Fuchs Uveitis Syndrome and Posner-Schlossman Syndrome

Fuchs uveitis syndrome, which was first described in the medical literature almost 200 years ago, presents a clinical picture of unilateral chronic AU; although variations are described, typical features include small "stellate" KPs diffusely distributed across the corneal endothelium, low-grade anterior chamber cell and flare, absence of posterior synechiae, iris atrophy that ultimately results in the appearance of iris heterochromia, anterior vitreous cells, and secondary cataract and glaucoma.[73] Recently confocal scanning laser ophthalmoscopy has expanded on these features, including identification of dendritiform and stippled KPs by standard scanning, and of abnormalities in iris autofluorescence by near-infrared scanning.[74, 75] Posner-Schlossman syndrome, or glaucomato-cyclitic crisis, also was first recognized by ophthalmologists many generations ago, as a unilateral acute recurrent AU with few KPs, low-grade anterior chamber cells and flare, and markedly elevated IOP.[76] More recent descriptions have highlighted the potential for progressive glaucomatous optic disc and visual field changes.[77] Almost simultaneously, infectious causes now have been assigned to both FUS and Posner-Schlossman syndrome. Rubella virus has been recognized as a cause of FUS, with epidemiological evidence from the United States showing a decline in incidence since the introduction of the rubella vaccination and an increase in the percentage of cases in foreign-born residents, and detection of RV in aqueous humor by GWC measurement and/or PCR.[5, 38, 39] Separately, PCR analyses in aqueous humor have identified CMV in patients previously diagnosed with Posner-Schlossman syndrome or FUS.[36] It is likely that other viruses may cause clinical pictures that suggest one of these syndromes, as exemplified by the report from India, of a patient with bilateral FUS, whose aqueous fluid tested positively for Chikungunya viral, but not RV, DNA by PCR.[48] Although it should be noted that concomitant involvement of rubella virus could not be excluded as GWC, which has a sensitivity of nearly 100%, compared to 10-20% for PCR, was not performed.

Immune Recovery Uveitis

Immune recovery uveitis (IRU) may present as an isolated anterior uveitis or more commonly with concurrent vitritis and cystoid macular edema, following immune recovery after highly active antiretroviral therapy in human immunodeficiency virus (HIV) patients or after tapering or discontinuation of immunosuppressive therapy in non-HIV patients with CMV retinitis. The condition represents an active immune response to CMV antigens that persist in the eye.[78-80] Posterior synechiae and posterior subcapsular cataract may develop, and after intraocular surgery, the postoperative course may be complicated by large inflammatory deposits on the surface of the intraocular lens.[78]

Laboratory Diagnosis Of Viral Uveitis

For the laboratory diagnosis of viral AU, one may perform blood analysis. However, serology at the most excludes a certain virus in the case of a negative result, or indicates whether a patient has ever been infected with the particular virus in the case of a positive result. In addition, most causes of viral AU have high seroprevalences in most parts of the world, particularly VZV and RV, rendering serology for these causes of little value. Polymerase chain reaction on peripheral blood is by no means conclusive, as negative results do not exclude an intraocular infection, and positive results do not prove one. A definitive diagnosis is only obtained by intraocular fluid analysis. Aqueous humor may be investigated by PCR or GWC analysis, to determine intraocular antibody production.[37, 38, 81]

Depending on the immune status, time of sampling and type of uveitis (chronic or (sub)acute), PCR or GWC analysis may be more sensitive. However, as these data may not always be available, it is advisable to perform both assays if possible.[82, 83]

Conclusions

Viral AU is typified by unilateral mild AU with fine or medium sized KPs, some form of iris atrophy, and sometimes high IOP. Cataract and glaucoma are common complications in viral AU and presence of vitritis in specific viral entities is common. There is an overlap in the clinical manifestations of AU caused by viruses and other non-viral forms of AU. Moreover, there is no specific feature that is indicative of viral AU, as many signs and symptoms may vary between specific viral causes (pain, redness, synechiae, corneal and vitreous involvement). Several viral AU entities may be accompanied by a prominent vitritis (eg. RV, HTLV and HIV), which can be misleading in making of correct diagnosis. FUS is usually also classified as AU, however vitreous involvement in FUS may be severe, and associated chorioretinal scars and papillitis also have been reported. It might be more correct to classify patients according to their actual presentations as intermediate or panuveitis.

In conclusion, a viral cause of AU should be suspected in patients with unilateral AU, exhibiting small or medium sized KPs, some form of iris atrophy, high IOP and early development of a cataract. Whilst medical history, serologic results and clinical features might raise a suspicion of viral etiology, the definitive diagnosis can be proven by aqueous humor analysis.

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chapter 5.2

Clinical Manifestations, Prognosis and Vaccination Status of Patients with Rubella Virus-Associated Uveitis

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Accepted for publication in American Journal of Ophthalmology

chapter 5.3

The Usefulness of Aqueous Fluid Analysis for Epstein-Barr Virus in Patients with Uveitis

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Ocul Immunol Inflamm. 2018; 00(00): 1–7

Abstract

Purpose To determine characteristics of patients with laboratory findings indicative of intraocular Epstein-Barr-Virus (EBV) infection and to establish the usefulness of the laboratory analysis in patients with uveitis.

Methods Retrospective study of patients who underwent diagnostic aqueous fluid analysis. Diverse demographic data of patients were registered.

Results EBV-PCR tested positive in 3/201 (1%) and EBV-GWC in 22/245 (9%). The prevalence of immunosuppression was similar in EBV positive (by PCR/GWC) and EBV negative patients (7/25; 28% vs. 50/272;18%, P=0.29). Out of all 22 EBV-GWC positive patients, GWC was between 3-10 in 91%. In total, 14 patients had laboratory results indicating only EBV infection. Patients without an alternative explanation for uveitis (6/14; 43%) had a chronic recurrent course and good visual prognosis.

Conclusion Low EBV-GWC values combined with multiple positive GWC and/ or PCR for other infectious agents. Intraocular assessment for EBV in the initial examination of uveitis patients has limited value.

Introduction

The association between uveitis and Epstein-Barr Virus (EBV) infection poses an enigma. Previous case reports and case series link EBV to various forms of uveitis, from bilateral granulomatous anterior uveitis to acute retina necrosis.[1-3]. Most reports based the association between uveitis and EBV infection on positive serologic results, suggesting concurrent active systemic EBV infection.[1,3,4]

Subsequently, more systematic reports emerged on this presumed association, reporting on polymerase chain reaction (PCR) positive for EBV in aqueous fluid of uveitis patients (up to 17%), however these positive PCR results were also found in uveitis of other established causes and even in non-uveitis eyes (7%), especially in patients with severe ocular disorders and break down of blood-retina barrier.[4-12] In one study examining the viral loads of EBV PCR positive patients, intraocular viral loads were always lower when compared to blood levels, which does not support the presumptive replication of EBV within the eye.[13,14]

Herein we report on a large series of uveitis patients who underwent diagnostic intraocular fluid assessment by both PCR and GWC for EBV in addition to Herpes Simplex Virus (HSV), Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), Rubellavirus (RV) and report on the clinical characteristics of patients with laboratory findings indicative of intraocular EBV infection.

Methods

Patients and data collection

All patients who underwent diagnostic aqueous fluid analysis between January 2010 and October 2016 at the Ophthalmology department of the Erasmus Medical Center (EMC, Rotterdam, the Netherlands) were included in this retrospective cohort study, which was approved by the Medical Ethics Committee and adheres to the Tenets of the Declaration of Helsinki (MEC-2012-016). We reviewed the medical records of all patients who had positive results in PCR or GWC for EBV.[15]

An aqueous fluid tap was performed in patients with a suspicion of infection (the presence of uveitis with or without small/ medium sized keratic precipitates (KPs), some form of iris abnormalities, high intraocular pressure (IOP) and resistance to steroids and non-conclusive results of initial uveitis work-up). Aqueous analysis was also performed before initiating systemic immunosuppressive treatment in patients with uveitis of unknown cause despite a standardized diagnostic investigation protocol (consisting of radiologic chest imaging, erythrocyte sedimentation rate, blood counts, serum angiotensin-converting enzyme levels, serology for syphilis as well as interferon gamma release assay (IGRA) test (QuantiFERON–TB Gold In-Tube test; (quantiferon; Cellestis Limited, Carnegie, Victoria, Australia)) and in those with anterior and panuveitis also Human Leukocyte Antigen B27 testing).

A diagnostic panel of PCR and GWC was determined in all diagnostic taps, which included assessment for HSV, VZV, CMV, RV and EBV. Additionally, quantitative EBV PCR analysis in peripheral blood was performed in the patients who tested positive by PCR for EBV in aqueous fluid.

In patients with laboratory indicators of EBV-associated uveitis, we registered diverse demographic and clinical data including gender, age at onset of uveitis, location and clinical features of uveitis and any systemic and ocular co-morbidity. The anatomical localization of uveitis was defined according to the Standardization of Uveitis Nomenclature.[16] The cause of uveitis, whenever known (and other than EBV) was also registered.

Sample collection and processing

The ocular fluid samples were stored at -80^{0}C and serum samples at +4^{0}C until processing for laboratory analysis. Determination of Intraocular Antibody Production: Specific immunoglobulin G (IgG) titers against RV, HSV, VZV, CMV and EBV in serum and aqueous humor were determined with the Euroimmun (Luebeck, Germany) indirect immunofluorescence test kit. The immunofluorescence assays (IFAs) are based on biochips, which were coated with

the virus specific-infected cells. Serial tenfold dilutions (1:10 to 1:5120) were prepared in sample buffer (Euroimmun). Samples were applied to the reaction fields of a reagent tray. After incubation for 30 min, slides were rinsed and immersed with phosphate-buffered saline (PBS). For detection of bound antibodies, slides were placed on reagent trays prepared with fluorescein conjugated anti-human immunoglobulin of the IgG class. Following a 30-min incubation, slides were washed as described above, embedded with mounting medium, cover slipped and evaluated by fluorescence microscopy.

IgG1 titres in serum and ocular fluid were determined using specific enzyme-linked immunosorbent assay (ELISA) kit (PeliClass human IgG subclass kit, Sanquin, Amsterdam, the Netherlands). The GWC was calculated as follows: GWC=((specific IgG eye/specific IgG serum)*(IgG1 serum/IgG1 eye)). Values exceeding 3 are considered indicative of intraocular antibody production.

Real-Time Taqman assay was performed as described previously.[17] For CMV, EBV, HSV1 and 2, rubella and VZV total nucleic acid was extracted from ocular fluid using the MagNaPure LC Total Nucleic Acid isolation kit (Roche, Almere, The Netherlands) with an input volume of 200µl (50µl of the ocular fluid sample was 4x diluted in RPMI-1640 (Lonza)) and output volume of 100µl. The extraction was internally controlled by the addition of a known concentration of phocine distemper virus (PDV) for RNA viruses and PhHV (Phocine herpes virus) for DNA virus.

Twenty µl extracted RNA was amplified in 50µl final volume, containing 12.5 µl 4 × TaqMan Fast Virus 1-Step Master Mix (including (1 U/µl) uracil-N-glycosylase, Life Technologies, Nieuwerkerk a/d IJssel, the Netherlands), and 1µl of a primers and probe mixture. For DNA viruses 5µl of trifluoroacetic acid (TFA) and 0,4µl of primers and probe mixture was amplified in a 20µl final volume. For CMV a dual target PCR was used.[17,18] For EBV, HSV1 and 2 and VZV primers were adapted from our earlier published procedure using real-time technique. Rubella RNA was amplified using forward primer (5'-cgtccagcaccctcacaag-3'), reverse primer (5'-cggagagttgccagacggt-3') and probe (FAM-cgtccgggtcagttccatacagaga-BHQ-1). The RT-PCR temperature profile was 5 min at 50°C, 20 sec at 95°C, 45 cycles of 3 s at 95°C and 30 sec at 60°C. Amplification was performed in an LC480 II(Roche Applied Science, Almere, the Netherlands) using the Fit Point analysis module. Quality assurance was performed using QCtoday software. The criterion for a successful RT-PCR run was that cycle threshold (Ct) values of both internal control and positive RT-PCR control should be within 3 × standard deviation (SD) of the mean.

Results

In total, 297 uveitis patients underwent an aqueous fluid tap out of which 201/297; 68% were tested for EBV-PCR and 245/297; 82% were tested for EBV-GWC (Table 1). Both assays were simultaneously performed in 184/297; 62% patients.

EBV-PCR tested positive in 3/201 (1%) and EBV-GWC in 22/245; 9%, resulting in 25 patients positive in intraocular fluid by at least one laboratory method for EBV. The total follow-up from aqueous fluid tap until last visit at our center of these patients was 2.5± 1.9 years). Out of these, 60% were of Caucasian origin and 64% were female. Further 28% were immunocompromised (immunosuppressive medication in 12% and human immunodeficiency virus (HIV)-positivity in 16%). The prevalence of immunosuppression was similar in EBV positive (either by PCR or GWC) and EBV negative patients (7/25; 28% vs. 50/272;18%, P=0.29, chi-square test). The mean age at onset of uveitis and distribution of anatomical localizations of uveitis was similar between EBV positive and EBV negative patients (Table 2).

The basic characteristics of patients positive for EBV PCR in intraocular fluid (N=3) are given in Table 3. Two of these three patients also tested positive by PCR for another infectious agent in aqueous and the clinical picture fitted the diagnosis of that particular infectious agent. The patient without any evidence of another infectious agent in PCR or GWC and no alternative diagnosis had bilateral multifocal choroiditis and was not immunocompromised. The blood sample of this patient was negative in EBV PCR (<100 IU/ml). One of these three PCR-positive patients in aqueous had also a EBV PCR positive blood sample, though with very low but detectable viral loads; this patient was immunocompromised by HIV infection (Table 3).

Twenty-two patients tested positive for EBV by GWC (Table 4). Out of these, 7 had multiple positive GWC's, 3 were positive by PCR for another infectious agent and 12 patients were positive only for EBV (Table 3). Out of all 22 EBV-GWC positive patients, GWC was between 3-10 in 91%. The two patients with higher GWC (≥10) were diagnosed with sarcoidosis (one of which was also HIV positive). The aqueous IgG titers for EBV were typically low, the exact titers in aqueous and serum are given in the supplementary Table. The majority of GWC positive patients 77% had another explanation of their uveitis than EBV. Out of these, 29% was caused by various infections and the remaining patients were diagnosed with associated non-infectious systemic diseases (mostly sarcoidosis, 29%).

In total, 14 patients had laboratory results indicating only EBV infection (either 1. positive EBV-PCR with negative results for PCR and/or GWC for other viruses or 2. a negative EBV- PCR but GWC positive for EBV and in cases with multiple positive coefficients, GWC for EBV had

the highest value). Out of these, 8 (57%) patients had another explanation for their uveitis. The GWC values of these patients were between 3-10 in 6 of 8 patients. No alternative explanation for uveitis was found in 6 (43%) patients. Three of these patients exhibited solely anterior chamber inflammation mostly with small KPs and marked involvement of the vitreous. Their vitritis was severe (requiring pars plana vitrectomy in two) but had no documented inflammatory involvement of the retina and/ or choroid.[16] The remaining three patients had solely anterior chamber inflammation without vitreous and/ or choroido-retinal involvement. All of these six patients had a chronic recurrent course of inflammations and good visual prognosis (all affected eyes had visual acuity at least of 20/20 at last follow-up. Only one of these six patients required systemic immunosuppressive treatment. The inflammation was bilateral in 4 of 6 patients and no other common characteristics were found. None of these 6 patients had aqueous fluid tap performed within 3 months after uveitis onset and their serum IgG levels for EBV were diverse (supplementary Table). None of the 25 patients PCR and/ or GWC positive patients for EBV had lymphoma at the onset of uveitis and/or was diagnosed with (intraocular) lymphoma during follow-up.

TABLE 1. Results of intraocular fluid analyses of 297 patients with uveitis.

	Positive PCR in tested patients	Positive GWC (≥3) in tested patients
Herpes Simplex Virus	10/271 (4%)*	1/257 (<1%)
Varicella Zoster Virus	9/271 (3%)	19/258 (7%)
Cytomegalovirus	12/248 (5%)	13/252 (4%)
Epstein-Barr Virus	3/201 (1%)	22/245 (9%)
Rubella Virus	9/183 (5%)	29/192 (15%)
Toxoplasma gondii	6/120 (5%)	12/106 (11%)

PCR = polymerase chain reaction, GWC = Goldmann- Witmer Coefficient.
**9/271 (3%) Herpes Simplex Virus type 1, 1/272 (<1%) Herpes Simplex Virus Type 2*

TABLE 2. Characteristics of patients tested for Epstein-Barr Virus in intraocular fluids.

	Total
Number	297
Age at onset uveitis *(mean years ±SD)*	46.4 (±18.8)
Gender	
Male	121/297 (41%)
Female	176/297 (59%)
Anatomical localization	
Anterior	97/297 (33%)
Intermediate	26/297 (9%)
Posterior	84/297 (28%)
Panuveitis	82/297 (28%)
Scleritis	8/297 (3%)

EBV= Epstein-Barr Virus PCR=polymerase chain reaction, GWC=Goldmann-Witmer Coefficient, SD=standard deviation.

TABLE 3. Ophthalmologic characteristics of patients positive in polymerase chain reaction for Epstein-Barr Virus.

	EBV PCR	EBV GWC	Other PCR+	Other GWC+	IS at moment of aqueous fluid tap	Laterality
Patient 1	+ *	-	-	-	-	2
Patient 2	+*	-	T. Gondii	-	-	1
Patient 3	+*	-	HIV-2	5.15 (CMV)	HIV+	1

EBV = Epstein-Barr virus, GWC = Goldman- Wittmer Coefficient, IS=Immunosuppression, KP = Keratic precipitates, AU=anterior uveitis, syn=synechia, MFC=multifocal chorioretinitis, CMV = Cytomegalovirus, HIV= Human Immunodeficiency Virus.

Positive GWC (≥3 but <10) in tested patients	Positive (GWC ≥10) in tested patients	Positive PCR and GWC (≥3.0) in tested patients
1/1 (100%)	0	0
11/19 (58%)	8/19 (42%)	7/245 (3%)
10/13 (77%)	3/13 (23%)	2/227 (1%)
20/22 (91%)	2/22 (9%)	0
8/29 (28%)	21/29 (72%)	7/167 (4%)
6/12 (50%)	6/12 (50%)	3/101 (3%)

PCR and/ or GWC negative	PCR positive for EBV	GWC positive for EBV
272/297 (92%)	3/297 (1%)	22/297 (7%)
46.9 (±18.8)	50.7 (± 11.8)	40.0 (± 17.5)
112/272 (41%)	1/3 (33%)	8/22 (36%)
160/272 (59%)	2/3 (67%)	14/22 (64%)
93/272 (34%)	0	4/22 (18%)
26/272 (10%)	0	0/22 (5%)
82/272 (30%)	1/3 (33%)	1/22 (5%)
65/272 (24%)	2/3 (67%)	15/22 (68%)
6/272 (2%)	0	2/22 (9%)

Localization	KPs	AU	Iris syn	Vitritis	Fundus	Alternative diagnosis
Panuveitis	+	+	+	+	MFC	None
Posterior	-	-	-	+	focal retinal lesion	Toxoplasmosis
Panuveitis	-	+	+	+	-	HIV-associated uveitis**

** The PCR for EBV in the serum of these patients was as following: negative (<100 IU/ml below the limit of detection, patient 1), negative (<100 IU/ml below the limit of detection, patient 2) and positive (<100 IU/ml detectable but below the limit of quantification, patient 3).*

*** The diagnosis of HIV-induced uveitis was made in this particular patient, as his intraocular HIV 2 loads were repeatedly higher then HIV-2 levels in plasma and uveitis subsided after the introduction of antiretroviral treatment.*

TABLE 4. Ophthalmologic characteristics of patients positive for Goldman-Wittmer Coefficient of Epstein-Barr Virus.

	EBV PCR	EBV GWC	Other PCR+	Other GWC+	IS at moment of aqueous fluid tap	Laterality
Patient 1	-	3.44	-	-	-	1
Patient 2	-	5.50	-	-	-	1
Patient 3	-	8.25	-	-	-	2
Patient 4	-	9.31	-	4.66 (VZV)	-	2
Patient 5	-	5.31	-	-	-	2
Patient 6	-	41.39	-	-	HIV+	1
Patient 7	-	3.74	-	-	-	2
Patient 8	-	7.86	-	-	-	2
Patient 9	-	9.29	-	-	Adalimumab + prednisolone	2
Patient 10	-	11.70	-	-	-	1
Patient 11	-	3.48	-	-	-	2
Patient 12	-	4.23	-	-	Adalimumab + methotrexate	2
Patient 13	-	4.29	-	-	-	2
Patient 14	0	3.63	+ (CMV)	-	HIV+	1
Patient 15	-	3.17	+ (Toxoplasmosis)	-	HIV+	1
Patient 16**	-	4.35	+ (RV)	-	-	1
Patient 17	-	4.07	+ (CMV)	260.5 (CMV)	Post kidney-transplantation	2
Patient 18	-	3.43	-	3.43 (HSV)	-	2
Patient 19	0	7.88	-	7.88 (VZV)	-	1
Patient 20	-	4.63	-	4.63 (VZV)	-	2
Patient 21	-	5.06	-	5.06 (VZV)	-	1
Patient 22**	-	4.64	-	5.90 (CMV)	-	1

EBV = Epstein-Barr virus, PCR=polymerase chain reaction, GWC = Goldman- Wittmer Coefficient, KP=Keratic precipitates, AU=anterior uveitis, syn=synechia, IS=Immunosuppression, HIV= Human Immunodeficiency Virus, POL = Punched Out Lesions, LTBI= Latent Tuberculosis Induced Uveitis, HLA-B27 = Human Leukocyte Antigen-B27, 0 = not performed, CMV = Cytomegalovirus,

Localization	KPs	AU	Iris syn	Vitritis	Fundus	Alternative diagnosis
Panuveitis	-	+	-	+	-	None
Anterior	-	+	-	-	-	None
Panuveitis	+	+	+	+	-	None
Anterior	-	+	-	-	-	None
Anterior	+	+	-	-	-	None
Panuveitis	-	-	-	+	POL Vasculitis	Sarcoidosis
Panuveitis	-	+	+	+	Granuloma's	Sarcoidosis
Panuveitis	+	+	-*	+	POL	Sarcoidosis
Panuveitis	+	+	-	+	Peripheral retinal scar	Sarcoidosis
Panuveitis	-	+	+	-	POL	Sarcoidosis
Panuveitis	+	+	-	+	-	LTBI-associated uveitis
Panuveitis	-	+	+	+	Vasculitis	HLA-B27+, psoritic arthritis, associated uveitis
Panuveitis	+	+	+	+	-	Multiple Sclerosis
Panuveitis	+	+	-	+	Occlusive vasculitis	CMV-associated uveitis
Panuveitis	+	+	+	+	Retinal detachment	Toxoplasmosis
Panuveitis	+	+	-	+	-	RV-associated uveitis
Posterior	-	-	-	+	CMV-retinitis	CMV-associated uveitis
Panuveitis	+	+	+	+	-	Multiple sclerosis
Scleritis	-	-	-	-	-	Varicella Zoster
Anterior	+	+	-	-	-	Kikuchi's disease
Scleritis	+	-	-	-	-	None, clinical suspicion Relapsing polychondritis
Panuveitis	-	+	+	+	-	None

RV = Rubellavirus, HSV = Herpes Simplex Virus, VZV = Varicella Zoster Virus, IgG = Immunoglobulin G.

**This patient had iris atrophy and iris nodules, without synechiae.*

***The aqueous humor tap that was positive for another viral agent than EBV was taken on another date than the aqueous humor tap being positive for PCR and/ or GWC EBV.*

Discussion

Our results show that EBV PCR and/or GWC can be detected in intraocular fluids of patients with uveitis of diverse origins and do not support a high prevalence of EBV-induced uveitis. Moreover, the positive EBV results of PCR and GWC in intraocular fluids were commonly combined with other positive results for infectious agents and the GWC levels were typically low.

In case series from 1990, EBV was considered as a possible cause of granulomatous anterior uveitis in a case series of 3 patients based on detectable IgG antibody titers against viral capsid antigen (VCA) in aqueous fluid. However, GWC was not calculated (but would have been <3.0 in 2 of these 3 patients) and PCR analyses for EBV were not performed.[1] Other reports supported the presumed association of EBV with uveitis by documenting positive serum and/ or aqueous fluid antibody levels, suggesting concurrent active systemic EBV infection. [1,3,4,19]

A more systematic study by Ongkosuwito et al, reported on the presence of EBV PCR in intraocular fluid (positive in 25/183;14% patients of uveitis) and GWC (positive in 3/82; 4%) in uveitis patients. Out of 25 EBV-PCR positive patients 9 (36%) were immunocompromised.[5] All three GWC positive patients did not match the clinical picture described in the initial case series (bilateral anterior granulomatous uveitis).[1,4,5] In addition, PCR positive for EBV was also detected in cataract controls (3/46; 7%) while GWC remained negative (none in 20 tested).[5]

Successive studies reported on positive EBV PCR patients and their intraocular loads, which were always lower when compared to blood. The only exception consisted of 2 patients with AIDS and primary central nervous system (CNS)/intraocular non-Hodgkin's lymphoma.[13,14] These previous findings show that intraocular replication of EBV in uveitis still remains to be proven. In addition, none of the patients exhibited simultaneous PCR and GWC for EBV.

Our study reports the results on simultaneous testing of EBV by PCR and GWC in 184 patients with uveitis. We noted a lower PCR yield for EBV (3/201; 1%) when compared to previous literature (up to 17%).[5,6,13,14] The prevalence of GWC was not systematically performed in the past except one study, which reports on 3/82; 4% prevalence of positive EBV GWC in uveitis patients (out of which 1 had a higher GWC for VZV), which is similar to 9% found in the present study.[5] It should be however noted that the GWC results in our study were typically low and/ or combined with multiple positive GWCs.

One explanation for the multiple positive GWCs might be a polyclonal stimulation of lymphocytes. In our series, one third of GWC positive patients had multiple positive coefficients (most commonly for VZV), which was also previously noted.[5,20-22] The other possibility might be the sensitivity of the GWC technique as the values for EBV were commonly low. The GWC is based on ratio of specific IgG levels in serum and aqueous and one should be aware of the caveats when interpreting the coefficient. Specifically, in low intraocular antibody titers for EBV (supplementary Table) one additional dilution step would result in a negative GWC value. Table 1 again illustrates this, showing that GWC for EBV having rather lower values in 91% of cases. This indicates that evaluation of the marginally positive GWC results should be carefully made and the exact levels of intraocular and serum antibodies should also be evaluated and included in the interpretation of GWC. Positive EBV PCR findings might be explained by migration of EBV infected lymphocytes into the eye. Additionally, the disruption of the blood-aqueous barrier might also play a role, especially in PCR positive cases. This phenomenon is supported by previous studies, in which PCR was more often positive for EBV in HIV positive patients with large areas of retinitis compared to cataract controls.[5,6] The common prevalence of immunosuppression (by HIV or immunosuppressive medication) in patients with positive PCR for EBV in intraocular fluids was made earlier.[6,13,14] In our study, solely 3 patients were PCR positive out of whom one was immunosuppressed; this limited number precludes any meaningful comparisons.

Our study describes 9% prevalence of low positive EBV GWC results but usually in combination with multiple positive GWC and/ or PCR for other infectious agents. Most patients had another explanation of uveitis and few patients had only EBV GWC as evidence for cause of their disease. Uveitis in the latter group was mostly nonspecific and had good visual prognosis. We conclude that performing intraocular assessment for EBV as part of an initial examination of intraocular fluids has limited value.

Supplementary Data

Supplemental Table 1 Results of Goldmann-Witmer Coefficient and Immunoglobuline G levels in serum and eye

https://doi.org/10.1080/09273948.2018.1543709

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chapter 6

Relevance of Erythrocyte Sedimentation Rate and C-Reactive Protein in Patients with Active Uveitis

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Graefes Arch Clin Exp Ophthalmol. 2019 Jan;257(1):175-180.

Abstract

Purpose To relate erythrocyte sedimentation rates (ESR) and C-reactive protein (CRP) values to different uveitis entities.

Methods A retrospective study of patients with a first episode of active uveitis visiting the Erasmus University Medical Center, uveitis clinic, Rotterdam, The Netherlands was performed. Levels of ESR and CRP were determined within 2 weeks and 1 week after onset of uveitis, respectively. Uveitis had to be of unknown origin at that moment. The specific etiologic groups were related to ESR and CRP values.

Results The majority of patients with uveitis had ESR and/or CRP values within the normal limits and no association of ESR and /or CRP with the specific cause of uveitis was observed. However, elevation of ESR≥ 60 mm/hour and/or CRP ≥60mg/L was mostly seen in patients with systemic immune-mediated diseases (8/59; 14% of all with immune mediated diseases) or systemic infectious causes (7/38;18% of all infectious uveitis). Patients with ocular toxoplasmosis typically exhibited normal ESR and CRP (9/11;82%) whilst patients with endogenous endophthalmitis had elevated ESR and/ or CRP in 6/7; 86%. Sarcoidosis-associated uveitis showed predominantly elevated ESR (13/24;54%; range 20-59 mm/hour in 11/13; 85%). Human Immunodeficiency Virus positive patients had more often elevated ESR values when compared to the remainder of patients (9/11; 82% vs. 64/163; 39%;18%, P=0.009). The cause of uveitis was established in 19/20 (95%) of patients with ESR≥ 60 mm/hour and/or CRP ≥60mg/L.

Conclusions The majority of patients with first attack of uveitis had ESR and CRP within the normal limits. Elevated levels of ESR and CRP reflected systemic involvement and high levels of both values were associated with established uveitis cause.

Introduction

Uveitis is an intraocular inflammation of multiple causes, which may result in permanent visual loss.[1-4] Erythrocyte sedimentation rate (ESR) together with C-reactive protein (CRP), both nonspecific markers of inflammation, are usually included in the initial diagnostic work-up. However, the clinical value of these parameters in the adult uveitis population is not known. Earlier investigations showed that ESR and CRP are within the normal range in a majority of patients with anterior uveitis.[5] In contrast, a recent report on juvenile idiopathic arthritis (JIA)-associated uveitis in a pediatric population showed that elevated ESR predicted the development of uveitis in patients with JIA.[6] However, it remains debatable whether ESR and CRP have any diagnostic value in evaluation of uveitis in adult patients having a first uveitis attack of unexplained origin.

Herein, we investigate the values of ESR and CRP during the first episode of active uveitis, determined within a short period after the onset in adult patients and relate the results to specific etiologic categories and clinical characteristics of uveitis.

Materials And Methods

The Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines were used to ensure the reporting of this observational study and this study followed the Tenets of the Declaration of Helsinki.[7]

We conducted a retrospective cross-sectional study at the ophthalmology department of the Erasmus Medical Center (Rotterdam, the Netherlands). All medical records of patients referred with new uveitis of unknown origin investigated between 2010-2017 were reviewed and 174 patients were identified who fulfilled our inclusion criteria. The onset of uveitis was defined as the first time active inflammation was documented by an ophthalmologist. ESR had to be determined ≤2 weeks and CRP values ≤1 week after the onset uveitis (as ESR normalizes within weeks and CRP levels within 7 days after resolution of tissue injury).[8] Exclusion criteria included age less than 18 years and patients with first mild anterior uveitis episode as these patients do not undergo diagnostic screening according to our guidelines. The ESR and CRP values were arbitrarily stratified to three subgroups (normal ESR <20 mm/hour, elevated ESR between 20 and 60 mm/hour, highly elevated ESR ≥60 mm/hour and normal CRP <10 mg/L (as defined in our laboratory), elevated CRP between 10 and 60 mg/L and highly elevated CRP ≥60 mg/L).

The following data were extracted from patients' records: age, gender, localization of uveitis, laterality, and human immunodeficiency virus (HIV) status. All immunosuppressive medications as well as co-morbidities were registered. Definitive anatomical classification was determined according to the Standardization of uveitis nomenclature (SUN) Working Group, by reviewing the whole follow-up period.[9]

The cause of uveitis was determined after the diagnostic examinations were completed. The diagnosis of definitive ocular sarcoidosis was given to patients that had histologically proven evidence and in all other cases, the criteria from the International Workshop On Ocular Sarcoidosis (IWOS) were used.[10] For the diagnosis of tuberculosis (TB)-associated uveitis a positive culture for mycobacteria in any fluid/ tissue sample was needed. Patients with a positive tuberculin skin test (Mantoux test) or interferon gamma release assay (IGRA) test with otherwise unexplained uveitis and no other indications of active tuberculosis were labeled as of unknown origin. All other specific diagnoses were performed according to current diagnostic criteria.[10-16]

All statistical analyses were performed using SPSS software (version 22.0, Chicago, IL, USA) and a P-value of <0.05 was considered statistically significant. Specific groups were categorized as mentioned above and compared with each other according gender, anatomical localization of uveitis, age and etiology. Continuous variables were described by mean and range, categorical variables with proportions and compared using the Mann-Whitney U test. Categorical variables were compared using the chi-square test or Fisher's exact test.

Results

The results of ESR and CRP measurements are shown in Table 1. Specific diagnoses in our cohort are depicted in the supplemental Table. A majority of patients was diagnosed with associated non-infectious systemic diseases (59/174; 34%) and had non-anterior uveitis (141/174; 81%). Slight female preponderance was observed (96/174; 55%). Immunosuppressive medication (required for other causes than uveitis) were used by 17/174; 10% patients. Patients suffering from diabetes mellitus (DM) and patients using immunosuppressive medication more often had elevated ESR values (P=0.018 for both, chi-square test), compared to the remainder of patients. No significant differences in ESR and CRP levels were found for gender, race, localization or laterality of uveitis (all p-values >0.05, chi-square test). Furthermore, elevated values of ESR and/ or CRP were not significantly associated with any of the etiologic categories.

Concordance and discrepancies between ESR and CRP are depicted in Table 2. A majority of patients had both ESR and CRP values within the normal limits (91/174; 52%). Elevation of only one of the parameters was seen in 50/174; 29%. Elevated levels of both parameters were found in 33/174; 19% patients.

The median ESR and CRP of patients with uveitis of established cause were higher than the median ESR and CRP of patients with unknown uveitis (17.0 mm/hour; range 1-120 mm/hour versus 11.0 mm/hour; range 1-140 mm/hour for ESR and 3.4 mg/L; range 0.4-262.0 mg/L versus 1.9 mg/L; range 0.3-229.0 mg/L, for CRP, P=0.015 for both, Mann-Whitney U test).

Out of 20 patients with either ESR ≥60 mm/hour and/or CRP ≥60mg/L, the cause of uveitis could be determined in 19/20. Fifteen had either noninfectious systemic disease or systemic infection, which was also a cause of uveitis (Table 3). The remaining 5 patients had uveitis limited to the eye, but had a concurrent systemic disorder, which explained their highly elevated ESR and/ or CRP but was not related to the cause of uveitis (such as multiple myeloma in a patient with infectious uveitis).

A majority of patients with infectious uveitis had ESR and CRP values within the normal limits (17/38; 45%) or only ESR ≥20 mm/hour (14/38; 37%), see Table 2. Discrepant results were more often noted in this group (17/38; 45% vs. 33/136; 24% P=0.024, chi-square test). Patients with toxoplasmosis exhibited normal ESR values in 9/11; 82% and all had CRP values within the normal limits. Patients with endogenous endophthalmitis exhibited CRP ≥10 mg/L, in 5/7; 71%.

Patients with non-infectious uveitis commonly had ESR and CRP values that were both within the normal limits (28/59; 47%; Table 2). If elevated (N=31), the parameters were most often elevated simultaneously (15/31; 48%). Of the patients with HLA B27- associated uveitis without systemic involvement, 2/8; 25% exhibited CRP ≥ 10 mg/L and 1/8; 13% had both ESR ≥20 mm/hour and CRP ≥10 mg/L. Two patients had HLA B27-associated uveitis with systemic involvement of which one had high elevation of CRP ≥60 mg/L and ESR ≥20 mm/hour and the other exhibited normal values. Sarcoidosis-associated uveitis showed a predominantly elevated ESR (13/24; 54%; in the range between 20-59 mm/hour 11/13; 85 %), whilst CRP was most often normal (17/24; 71%). Systemic involvement in sarcoidosis patients (hilar lymphadenopathy as seen on chest imaging) was present in 21/24 (88%), however, in only 4/21 (19%) of these, treatment was required.

The HIV was positive in 11/62; 18% tested patients. HIV positive patients had more often elevated ESR values when compared to the remainder of patients (9/11; 82% vs. 64/163; 39%, P=0.009, chi-square test). An infectious cause for uveitis was found in 9/11;82% HIV positive patients, out of which 4/9;44% had CMV retinitis and 4/9;44% had syphilitic uveitis. ESR ≥60 mm/hour together with HIV-positivity was observed in 4/11;36% (2 with CMV retinitis, one with syphilitic uveitis and one with sarcoidosis-associated uveitis). Only one of the HIV positive patients exhibited normal values of both ESR and CRP, this patient was diagnosed with CMV retinitis.

TABLE 1. Erythrocyte sedimentation rate and C-reactive protein of patients with a first episode of active uveitis of unknown cause.

	Total N=174	**ESR[a]**
		<20 (N=101)
Total	174	101/174 (58%)
Diabetes mellitus	17/174 (10%)	5/17 (29%)
Immune suppressive medication[b]	17/174 (10%)	5/17 (29%)
Human Immunodeficiency Virus positivity [c]	11/174 (6%)	2/11 (18%)
Anatomical localization		
Anterior	33/174 (19%)	18/33 (55%)
Intermediate	2/174 (1%)	1/2 (50%)
Posterior	45/174 (26%)	32/45 (71%)
Panuveitis	86/174 (49%)	48/86 (56%)
Scleritis	8/174 (5%)	2/8 (25%)
Non-infectious systemic disease	**59/174 (34%)**	**33/59 (56%)**
Infectious uveitis	**38/174 (22%)**	**20/38 (53%)**
Established clinical entity	**24/174 (14%)**	**14/24 (58%)**
Unknown	**53/174 (30%)**	**34/53 (64%)**

ESR = erythrocyte sedimentation rate, CRP = C-reactive protein.

[a] ESR had to be determined <2 weeks of onset, CRP within <1 week of onset.

ESR[a]			CRP[a]	
20-59 (N=57)	**≥60 (N=16)**	**<10 (N=131)**	**10-59 (N=33)**	**≥60 (N=10)**
57/174 (33%)	16/174 (9%)	131/174 (75%)	33/174 (19%)	10/174 (6%)
9/17 (53%)	3/17 (18%)	12/17 (71%)	5/17 (29%)	0
7/17 (41%)	5/17 (29%)	12/17 (71%)	2/17 (12%)	3/17 (18%)
5/11 (45%)	4/11 (36%)	10/11 (91%)	0	1/11 (9%)
10/33 (30%)	5/33 (15%)	23/33 (70%)	8/33 (24%)	2/33 (6%)
1 (50%)	0	2/2 (100%)	0	0
12/45 (27%)	1/45 (2%)	37/45 (82%)	8/45(18%)	0
30/86 (35%)	8/86 (9%)	65/86 (76%)	15/86(17%)	6/86 (7%)
4/8 (50%)	2/8 (25%)	4/8 (50%)	2/8 (25%)	2/8 (25%)
20/59 (34%)	**6/59 (10%)**	**39/59 (66%)**	**14/59 (24%)**	**6/59 (10%)**
11/38 (29%)	**7/38 (18%)**	**31/38 (82%)**	**4/38 (11%)**	**3/38 (8%)**
8/24 (33%)	**2/24 (8%)**	**17/24 (71%)**	**7/24 (29%)**	**0**
18/53 (34%)	**1/53 (2%)**	**44/53 (83%)**	**8/53 (15%)**	**1/53 (2%)**

[b] *Indicated for other causes than uveitis.*

[c] *HIV was tested in 62 patients, out of which 11/62 (18%) were found positive.*

TABLE 2. Concordance and discrepancies in erythrocyte sedimentation rate and C-reactive protein in patients with uveitis.

	Total
	N = 174
Age at onset of uveitis (years)	
Mean (±SD)	45.8 *(±17.1)*
Localization	
Anterior Uveitis	*33/174* (19%)
Intermediate uveitis	*2/174 (1%)*
Posterior uveitis	*45/174 (26%)*
Panuveitis	*86/174 (49%)*
Scleritis	*8/174 (5%)*
Laterality	
Unilateral	83/174 (48%)
Bilateral	91/174 (52%)
Gender	
Females	96/174 (55%)
Males	78/174 (45%)
Race	
Caucasian	110/174 (63%)
Non-Caucasian	64/174 (37%)
Non-infectious systemic disease	**59/174 (34%)**
Sarcoidosis[b]	*24/59 (41%)*
HLA B27-associated uveitis	10/59 (17%)
Miscellaneous[c]	25/ 59 (42%)
Infectious	**38/174 (22%)**
Toxoplasmosis	11/38 (29%)
Endogenous endophthalmitis	7/38 (18%)
Miscellaneous[d]	20/38 (53%)
Established clinical entity[e]	**24/174 (14%)**
Unknown	**53/174 (30%)**

ESR = erythrocyte sedimentation rate, CRP = C-reactive protein, SD = standard deviation, HLA B27 = Human Leukocyte Antigen B27.

[a] *ESR had to be determined <2 weeks of onset, CRP within <1 week of onset.*

[b] *17/24 (71%) of sarcoidosis patients was biopsy confirmed.*

[c] *Including patients with Vogt-Koyanagi-Harada syndrome (N=6), multiple sclerosis (N=4), Behçet's Disease (N=3), inflammatory bowel disease (N=3), granulomatosis with polyangitis (N=2), reactive arthritis with uveitis (N=2), acute disseminated encephalomyelitis (N=1), Kikuchi disease (N=1), relapsing polychondritis (N=1), systemic lupus erythematosus (N=1), systemic vasculitis not otherwise specified (N=1).*

	Concordant results ESR and CRP[a]		Discrepant results ESR and CRP[a]	
	CRP <10 mg/L and ESR <20 mm/hour N= 91	**ESR ≥20 mm/hour and CRP ≥10 mg/L N = 33**	**ESR ≥20 mm/hour but CRP <10 mg/L N=40**	**CRP ≥10 mg/L but ESR <20 mm/hour N= 10**
	43.1 *(±17.3)*	45.9 *(±15.6)*	51.7 *(±15.9)*	46.6 *(±20.7)*
	16/33 (48%)	*8/33 (24%)*	*7/33 (21%)*	*2/33 (6%)*
	1/2 (50%)	*0*	*1/2 (50%)*	*0*
	29/45 (64%)	*5/45 (11%)*	*8/45 (18%)*	*3/45 (7%)*
	44/86 (51%)	*17/86 (20%)*	*21/86 (24%)*	*4/86 (5%)*
	1/8 (13%)	*3/8 (38%)*	*3/8 (38%)*	*1/8 (13%)*
	43/83 (52%)	*15/83(18%)*	*20/83 (24%)*	*5/83 (6%)*
	48/91 (53%)	*18/91 (20%)*	*20/91 (22%)*	*5/91 (5%)*
	50/96 (52%)	*16/96 (17%)*	*25/96 (26%)*	*5/96 (5%)*
	41/78 (53%)	*17/78 (22%)*	*15/78 (19%)*	*5/78 (6%)*
	59/110 (54%)	22/110 (20%)	23/110 (21%)	6/110 (5%)
	32/64 (50%)	11/64 (17%)	17/64 (27%)	4/64 (6%)
	28/59 (47%)	**15/59 (25%)**	**11/59 (19%)**	**5/59 (8%)**
	10/24 (42%)	6/24 (25%)	7/24 (29%)	1/24 (4%)
	6/10 (60%)	2/10 (20%)	0	2/10 (20%)
	12/25 (48%)	7/25 (28%)	4/25 (16%)	2/25 (8%)
	17/38 (45%)	**4/38 (11%)**	**14/38 (37%)**	**3/38 (8%)**
	9/11 (82%)	0	2/11 (18%)	0
	1/7 (14%)	2/7 (29%)	1/7(14%)	3/7 (43%)
	7/20 (35%)	2/20 (10%)	11/20 (55%)	0
	13/24 (54%)	**6/24 (25%)**	**4/24 (17%)**	**1/24 (4%)**
	33/53 (62%)	**8/53 (15%)**	**11/53 (21%)**	**1/53 (2%)**

[d]*Including varicella-zoster virus (N=5), cytomegalovirus (N=4), syphillis (N=4), herpes simplex virus (N=3), rubella virus (N=2), bartonella (N=1), tubercdulosis (N=1).*

[e] *Including patients with acute multifocal posterior placoid pigment epitheliopathy (N=3), birdshot chorioretinopathy (N=2), toxic uveitis (N=2), post-traumatic uveitis (N=2), sympathetic ophthalmia (N=1), serpiginous choroidopathy (N=1), purtscher like retinopathy (N=1), Fuchs heterochromic uveitis syndrome (N=1), punctate inner choroidopathy (N=1). The masquerade syndromes including lymphoma (N=3), macular drusen (N=2), human immunodeficiency virus related microangiopathy (N=1), macular dystrophy (N=1), uveitis suspected to be caused by bacillus Calmette-Guérin intravesical immunotherapy for bladder cancer (N=1), cotton wool spots (N=1), Coats'disease (N=1)*

Discussion

In this retrospective, cross-sectional study, the majority of patients with a first active episode of uveitis of unknown origin presented with normal ESR and CRP values. Moreover, no significant relationship between the levels of these biomarkers and specific causes of uveitis was found.

Earlier investigations of ESR and CRP in uveitis patients also demonstrate normal values in a majority of patients with anterior uveitis, but none of these previous studies defined the time window in which ESR and CRP were determined in relation to the onset of uveitis, while both biomarkers are susceptible for changes within short periods.[5, 8, 17] Biomarkers like ESR and CRP are commonly assessed during the diagnostic work-up of new uveitis patients for potential detection of infections or systemic immune-mediated disease causing uveitis.[1-4, 18-21] Elevated levels of ESR are due to a higher plasma protein levels (e.g. fibrinogen, gamma globulins) and CRP is an acute phase protein released after tissue injury caused by infections or other sources of inflammation.[8, 22]

Though a majority of patients with infectious uveitis in the present study exhibited normal ESR and CRP values, increased values were predominantly encountered in systemic infections. The high levels of ESR and CRP were found in patients with endogenous endophthalmitis, an ocular inflammation that occurs concurrently with bacteremia. In contrast, these inflammatory parameters were nearly always normal in patients with ocular toxoplasmosis, an intracellular parasite. Reactivation of these dormant parasites within the eye is not being accompanied by any systemic activity.

In HIV patients, the common hyperimmunoglobulinemia causes elevation of ESR (rather than a direct infectious trigger causing release of fibrinogen). In our series, highly elevated ESR was often seen in HIV-positive and therefore it might be therefore worthwhile to determine HIV status in patients with uveitis of unknown origin and unexplained high ESR.[23] Patients using immunosuppressive medications and those suffering from DM had more often elevated ESR values and the yield of these tests is therefore lower in these patients.

Elevated levels of ESR and CRP are common in systemic sarcoidosis patients, specifically in sarcoidosis-associated arthritis and erythema nodosum compared to other clinical presentations.[24, 25] Half of the patients with ocular sarcoidosis, however, had both normal ESR and CRP, which might reflect mild (or lack of) systemic involvement at the moment of onset of first uveitis attack.[26] This is also illustrated in the current series, where the majority of patients had very mild extraocular involvement which might also explain their predominantly normal ESR and CRP values.

In this study, follow-up measurements of ESR and/ or CRP were not available and consequently we do not have information about the highest levels of ESR and CRP reached in individual patients. Including the highest levels of ESR and CRP could possibly expose some associations, which were not found in the present study. In addition, the changes of ESR and/or CRP might change in individual patients and might be associated with impeding uveitis activity. However, our main goal was to determine the diagnostic value of these parameters during the first stage of uveitis after presentation. Data on body mass index, which can influence the ESR and CRP values, were not available in our patients.[27]

In conclusion, our study reflects that presence of uveitis alone is not sufficient to cause elevation of ESR and/ or CRP as a majority of patients with a first uveitis episode had ESR and CRP values within the normal limits. In patients with highly elevated ESR and/ or CRP, the presence of a systemic disease is very likely and in consequence, the cause of uveitis is being established in a vast majority of cases.

Supplementary Data

Supplemental Table Demographics and baseline characteristics of patients with active uveitis of unknown cause at onset**.**

https://doi.org/10.1007/s00417-018-4174-7

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chapter 7

General Discussion and Summary

chapter 7.1

General Discussion and Summary

Summary

Uveitis is caused by diverse infectious and non-infectious systemic diseases. The initial diagnostic work-up of uveitis patients is designed to differentiate between common infectious and non-infectious causes, which is crucial for timely treatment of curable infections. However, even after a careful diagnostic evaluation of patients, approximately 30-40% of uveitis cases remain without an established cause. The Dutch national guidelines for uveitis recommend to initiate investigations according to the anatomical localization of uveitis. However, the actual diagnostic value of some of these tests is not known. The objective of the research presented in this thesis is to evaluate various diagnostic techniques used in daily uveitis practice and to report on the outcome of patients tested positive with some of these investigations.

In **Chapter 2** we provided a general overview of the ocular morbidity and visual outcome of newly referred patients to a tertiary uveitis center. We performed a retrospective cohort study of 133 patients (219 affected uveitis eyes) and followed this population during one year. The visual prognosis of uveitis patients was favorable at one-year follow-up with bilateral visual impairment in only 5/133; 4% of patients. At least one ocular complication developed in 88/133; 66% of patients and 40/133; 30% of patients required at least one intraocular surgery, mostly cataract extraction (30/51; 59% of all operations). Moreover, systemic immunosuppressive treatment was required in 47/133; 35% of patients and the mean number of visits to an ophthalmologist was 11 per year while 8% patients required hospital admission. Visual impairment was mostly due to cystoid macular edema, retinal scars, and glaucoma. Prognosticators for poor visual outcome included visual impairment at referral (odds ratio [OR], 23; 95% confidence interval [CI], 9-63; P <0.001), and glaucoma before referral (OR, 28; 95% CI, 8-100; P <0.001). The course of uveitis was however variable and visual performance changed according to its activity. The visual acuity at one time point therefore did not reflect the burden of visual impairment. The mean duration of visual impairment in the first year after referral was 4 months per eye. Our results illustrate a favorable visual prognosis in the first year after referral despite severe and multiple ocular complications that needed frequent visits to an ophthalmologist and commonly required intraocular surgery.

Chapter 3.1 was devoted to the ophthalmologic characteristics seen in sarcoidosis. We summarize the current knowledge regarding the ocular involvement in sarcoidosis. Ophthalmologic involvement is present in approximately 40% of patients with pulmonary sarcoidosis and the eye is the presenting organ in approximately 20% of sarcoidosis patients. Involvement of the lacrimal gland and conjunctiva is frequent, presenting usually with dry eyes complaints and has good visual prognosis. Uveitis is the most common ophthalmologic manifestation and often shows a chronic course in patients with sarcoidosis. Characteristic

clinical presentation of sarcoidosis-associated uveitis includes a painful bilateral anterior granulomatous uveitis, which develops often in black patients of young age, whilst a painless bilateral posterior uveitis with peripheral multifocal choroiditis manifests mostly in older, white females. Optic nerve involvement is rare, but important to recognize because it is associated with a poor visual prognosis and requires systemic treatment. Local treatment with steroid drops and/or periocular injections is the first step of treatment whilst systemic treatment is primarily indicated in patients with risk of visual deterioration and/or optic nerve involvement. The diagnosis is mostly substantiated by criteria of the 'International Workshop on Ocular Sarcoidosis' (IWOS), but these criteria still need validation in clinical practice. Ocular sarcoidosis with its wide spectrum of presentation should be included in the differential diagnosis of all uveitis patients.

Chapter 3.2 was a cross-sectional retrospective study evaluating the diagnostic value of chest radiographs in 200 patients with recent-onset uveitis (less than 1 year) of undetermined cause. The chest X-ray had to be performed during the initial diagnostic evaluation, before any systemic treatment was initiated. Chest computed tomography (CT) imaging and somatostatin receptor scintigraphy were performed if the initial diagnostic evaluation raised a suspicion of sarcoidosis. Chest radiographic findings consistent with sarcoidosis were defined as symmetrical bilateral hilar lymphadenopathy and/ or suggestive interstitial lung patters for sarcoidosis. Presumed sarcoidosis was defined as absence of biopsy-confirmed disease but typical findings on imaging (chest X-ray, chest-CT or somatostatin receptor scintigraphy). Patients with unexplained uveitis, normal imaging and elevated angiotensin converting enzyme (ACE) were diagnosed as uveitis of unknown origin. In total, 30/200; 15% of chest radiographs were abnormal and suggested mostly the presence of sarcoidosis (13/30; 43%). The sensitivity and specificity of chest radiograph for the diagnosis of biopsy-proven sarcoidosis in uveitis patients was 64% and 91%, respectively. The sensitivity and specificity of elevated ACE for biopsy-proven sarcoidosis was 41% and 93%, respectively. The combined sensitivity and specificity of chest radiograph and ACE for biopsy proven sarcoidosis was 79% and 84%, respectively. Based on these findings, we conclude that the current strategy to detect sarcoidosis in uveitis patients has good diagnostic value, but there certainly is more space left for better performing diagnostic tests.

In **Chapter 3.3** we compared the diagnostic value of soluble Interleukin 2 receptor (sIL-2R) and ACE for the diagnosis of sarcoidosis-associated uveitis. The serum sIL-2R is a protein released from activated T cells and therefore a surrogate marker for T cell activation. In contrast, the elevated ACE levels in sarcoidosis are attributed to increased production by the epithelioid cells present in the sarcoid granuloma. In patients with systemic sarcoidosis, sIL-2R levels are associated with disease activity, but the diagnostic value of this test in uveitis patients was not known. We conducted a retrospective cross-sectional study of 249

consecutive uveitis patients in a tertiary center. Although sarcoidosis-associated uveitis patients had the highest mean serum sIL-2R (6047 pg/mL) and ACE (61 U/L) levels, elevated serum sIL-2R levels are also observed in patients with HLA-B27 associated uveitis (4460 ±2465 pg/mL) and VZV- associated uveitis (5386 ±1778 pg/mL). The Receiver Operating Characteristic (ROC), was slightly higher for sIL-2R when compared to ACE (0.76 vs. 0.65, P=0.06). The highest Youden index for sIL-2R was 0.45, which yielded an optimal cut-off of 4000 pg/mL. The corresponding optimal sensitivity and specificity of sIL-2R for the diagnosis of sarcoidosis were 81% and 64%, respectively. The highest Youden-index was clearly lower for ACE (0.23) when compared to sIL-2R. The sensitivity and specificity of ACE are 30% and 85%, respectively. The combination of sIL-2R and chest radiograph resulted in a sensitivity and specificity of 92% and 58%. The combined sensitivity and specificity of ACE and chest radiograph in this series was 70% and 79%. We conclude that for the diagnosis of sarcoidosis-associated uveitis, sIL-2R had slightly higher diagnostic value than ACE, especially considering the sensitivity.

Lymphopenia (<1.5x10^9/L) was often noticed in sarcoidosis patients. In **Chapter 3.4** we investigated the diagnostic value of lymphopenia for the diagnosis of sarcoidosis-associated uveitis in untreated patients with a first uveitis episode. We conducted a retrospective cross-sectional study in patients with uveitis who did not receive any systemic immunosuppressive treatment and any other possible cause of lymphopenia (N=53) was excluded. Lymphocyte counts had to be determined within one month of the first period of uveitis. Lymphopenia was observed in 61/191; 32% of uveitis patients, mostly in noninfectious systemic diseases associated with uveitis (32/65; 49%), particularly in sarcoidosis (24/32;75%). In other systemic diseases, related to uveitis, lymphopenia occurred significantly less frequent (e.g. Human Leukocyte Antigen (HLA)-B27- associated uveitis, Behçet's disease, multiple sclerosis, inflammatory bowel disease, granulomatosis with polyangiitis and Vogt-Koyanagi-Harada syndrome). However, lymphopenia was also observed in patients with uveitis of unknown origin (21/73; 29%). Patients with lymphopenia had a 12-fold higher chance of having sarcoidosis (95% confidence interval; 4.7-30.5), corrected for sex, race and age. The negative predictive value (NPV) of lymphopenia for the diagnosis of sarcoidosis-associated uveitis was 94%, which is similar to the NPV of chest radiograph (95%) and sIL-2R (95%) noted in previous reports. The NPV of lymphopenia was higher than that of ACE (87%). Normal lymphocyte counts might be helpful in excluding sarcoidosis as a cause of uveitis.

In **Chapter 4**, we determine the prevalence and clinical implications of positive Quantiferon-Gold (QFT-G) test results in the diagnostic work-up of a large cohort of patients with uveitis in the Netherlands by means of a retrospective cross-sectional study of patients with uveitis who underwent QFT-G testing. Out of all 710 patients, 92 (13%) tested positive for QFT-G, whilst prior TB was only documented in 2 patients. Active, culture proven TB was observed

only in one case. Out of all 92 QFT-G positive patients, 54/92 (59%) had uveitis of not established origin and 12 (13%) were diagnosed with (presumed) TB and/or sarcoidosis; the remaining 26 (28%) had uveitis of recognized origin, but not related to their QFT positive results. The proportion of patients with uveitis of unknown etiology was higher in QFT-G positive than in the QFT-G negative patients (P=0.000). Twenty-nine of QFT-G positive patients with otherwise unexplained uveitis completed anti-tuberculous therapy (29/710; 4% of all included patients) with beneficial effect in the majority of cases. The uveitis features of these QFT-G positive patients were mainly nonspecific. Out of all QFT-G positive patients with uveitis, 17 patients had chest-imaging changes suggesting either TB or sarcoidosis. We conclude that QFT-G testing is useful in the work-up for uveitis in the Netherlands as the QFT-G positive patients with uveitis of otherwise unexplained origin might profit from ATT, especially those with severe and sight threatening uveitis.

Chapter 5.1 described the typical manifestations of viral anterior uveitis (AU) entities. The viral causes of AU emerged with the use of novel molecular diagnostic and serologic tests adapted for small volumes (polymerase chain reaction; PCR and Goldmann-Witmer Coefficient; GWC). The most common AU-inciting viral infections and associated systemic diseases were included in this review. We describe distinctive signs for individual viruses and attempt to discriminate which clinical presentations should raise a suspicion of viral etiology. We conclude that viral AU is commonly characterized by unilateral AU with fine or middle-sized keratic precipitates (KPs), some form of iris atrophy, elevated intraocular pressure and early development of cataract. Some ocular characteristics are more specific for individual viruses such as a prominent vitritis was typical for Rubella Virus (RV)-associated uveitis and Human T-lymphotropic-Virus type 1. The absence of synechiae was characteristic of RV-associated uveitis whilst Varicella-Zoster Virus (VZV)-associated uveitis and Herpes Simplex Virus (HSV)-associated UA typically showed posterior synechiae. Additionally, VZV-associated uveitis classically presented in older patients with characteristic skin involvement. Sectorial iris atrophy was typical for HSV-associated AU. A differentiating feature of cytomegalovirus (CMV)-associated AU was corneal endotheliitis together with coin-like KP's and a mild AU. Moreover, this virus might also cause clinical entities entitled previously as Possner- Schlossman syndrome or Fuchs Uveitis Syndrome (FUS). The definitive diagnosis of a viral etiology in AU should be confirmed with intraocular fluid analysis by PCR and/ or GWC.

In **Chapter 5.2**, we investigated the clinical spectrum of RV-associated uveitis. To date, many clinicians have assumed that most cases with RV-associated uveitis present as FUS (a constellation of following ophthalmologic characteristics: chronic AU, diffuse iris atrophy, KPs, absence of posterior synechiae and the early presentation of cataract). In this retrospective cohort study, we investigated 127 RV-associated uveitis patients, proven with intraocular fluid

analysis (by PCR and/ or GWC) and their ophthalmologic characteristics and complications. We found that only a minority of RV-associated uveitis patients manifested as FUS (37/127; 29%). In addition, during the same study period, 39 patients presented with complete FUS to our center, of which only 2/39; 5% were negative for RV in intraocular fluid analysis. The most common combination of ophthalmologic characteristics in RV-associated uveitis consisted of unilateral AU with vitritis in the absence of posterior synechiae and CME. Cataract developed in nearly all patients (101/127; 80%) and half of patients developed elevated intraocular pressure and/ or glaucoma during a median follow-up of 3.1 years (Interquartile Range 7.7). None of the unilateral cases developed involvement of the other eye during follow-up. None of the patients with RV-associated underwent RV vaccination in childhood. We show that whilst FUS is often caused by RV, RV-associated uveitis has a much wider spectrum of ocular manifestations and presents with a FUS phenotype only in a minority of patients. Our results emphasize the need for long-term monitoring of intraocular pressure in these patients and show that bilateral involvement is already present at the onset of the disease. Glaucoma developed more frequently in RV PCR positive patients, which may be of use to an uveitis expert in his or her prognostic consideration in an individual uveitis patient. However, the exact strength of this associations is challenging to investigate as the time of uveitis onset is often insidious in these patients. Future research should analyze the time to glaucoma development from onset of uveitis and their PCR positivity.

The presumed association between Epstein-Barr Virus (EBV) infection and uveitis was repeatedly proposed, but the true relationship still imposes an enigma. Infection with EBV is almost universal as a large proportion of adult population is seropositive in for this virus. In **Chapter 5.3** we investigated a large series of uveitis patients who underwent diagnostic intraocular fluid assessment by both PCR and GWC for EBV and other viruses and described the clinical picture of patients with laboratory results suggesting EBV infection. Only 3/201; 1% and 22/245; 9% patients were positive for EBV by PCR and GWC, respectively. None of these 25 patients had lymphoma at moment of aqueous fluid tap, nor developed this condition during the follow-up (2.5 ± 1.9 years). The GWC was between 3-10 in 20/22; 91% of patients and 10/22; 45% of EBV GWC positive patients had multiple positive results for infectious agents in intraocular fluid analysis. Laboratory results indicating only EBV infection were documented in 14/25; 56%, but out of these a majority (8/14; 57%) had a proven alternative diagnosis. A positive intraocular fluid analysis indicating only EBV as a possible cause of uveitis was determined in only 6/25; 24% patients who had no alternative diagnosis. Uveitis in these patients had mainly a good visual prognosis with chronic recurrent course and no characteristic ophthalmologic findings. We conclude that performing intraocular fluid analysis for EBV as part of initial examination of intraocular fluid has limited value. The possibility of EBV-driven uveitis or EBV lymphoma might however exist in immunocompromised patients. The diagnostic value of two inflammation markers, namely erythrocyte sedimentation rate

(ESR) and c-reactive protein (CRP) was evaluated in patients with recent, first uveitis episode in **Chapter 6** A cross-sectional study was performed in 174 patients, who had ESR performed within 2 weeks and CRP within 1 week of uveitis onset. The majority of patients (91/174; 52%) had normal ESR (<20 mm/hour) and CRP (<10 mg/L) values. Patients with infectious uveitis showed normal ESR and CRP values in 17/38; 45% and elevated values were mostly observed in systemic infections, such as endogenous endophthalmitis (5/7; 71%). Normal ESR and CRP values in infectious uveitis were mainly found in local intraocular infections such as toxoplasmosis (9/11; 82%). Out of 20 patients with ESR ≥60 mm/hour and/ or CRP ≥60mg/L, 15/20; 75% had a (non) infectious systemic disease related to uveitis explaining the high values of these examinations and 5/20; 25% had another explanation for highly elevated parameters such as multiple myeloma, sepsis or severe infection following an extensive surgery. No association between elevated ESR and/ or CRP values and a specific etiologic cause of uveitis was found. We conclude that elevated ESR and/ or CRP values do not have any differentiating capacity for the cause of uveitis, but reflect systemic activity of inflammation.

General Discussion And Future Perspectives

The main objective of this thesis was to investigate the utility of various diagnostic tests in the work-up of patients with uveitis. Currently, the diagnostic work-up of uveitis patients is directed to a quick identification of curable infectious uveitis entities, together with a focus to detect the most common associated noninfectious causes. This thesis contains practical information on the utility of diverse diagnostic tests in uveitis with the emphasis on ocular sarcoidosis and diverse infectious uveitis entities.

Sarcoidosis

Sarcoidosis is one of the major causes of uveitis in Europe. The presence of non-caseating granuloma in a biopsy of relevant tissue is required to diagnose sarcoidosis, in conjunction with exclusion of other granulomatous diseases.[1] However, biopsy of intraocular tissue is an invasive procedure and not readily performed. In the frequent absence of histologic evidence, indirect testing dominates the diagnostic work-up of uveitis. In clinical practice, uveitis together with histologic evidence of sarcoidosis in another organ (e.g., skin, lung) would generally be sufficient to a make a diagnosis of sarcoidosis-associated uveitis. Non-directed conjunctival and lacrimal gland biopsies were found not useful in uveitis patients, but their exact value remains a matter of debate.[2-7] Patients with sarcoidosis-associated uveitis showed elevated intraocular ACE levels in one series, but this finding was so far not confirmed by others.[8] An elevated CD4+/CD8+ lymphocyte ratio of vitreous and aqueous humor was found specific for ocular sarcoidosis in previous reports, but also in proliferative diabetic retinopathy. The diagnostic value in clinical practice remains to be investigated.[9-12]

The IWOS criteria for the diagnosis of ocular sarcoidosis were established in 2009, in order to make a presumptive diagnosis of sarcoidosis in patients without histologic evidence of disease.[13] These criteria are based on ophthalmic signs, laboratory and/ or imaging findings (i.e. bilateral hilar lymphadenopathy). The Dutch uveitis guideline recommends chest radiography and determination of serum ACE levels in every patient with uveitis (except for the first, mild anterior uveitis and children).[14]

Predictive value of diagnostic tests for sarcoidosis

One should realize that screening tests do not diagnose a disease but rather select patients who test positive and require further evaluation with subsequent diagnostic tests. A high PPV value indicates that most patients with a positive test do have the disease and ideally this should be also combined with a high NPV. These probability values are most important to a uveitis specialist because further clinical decisions considering other diagnostic tests or treatment are based on test results.

Prevalence of sarcoidosis

For interpretation, predictive values have to be weighed against the (pre-test) chance of having or not having the disease. The prevalence of sarcoidosis in the uveitis population in northern Europa is approximately 10%, so the chances of not having sarcoidosis are around 90%.[15-22] In general, all three sarcoidosis biomarkers investigated in this thesis (ACE, sIL-2R and lymphocyte counts), had high NPVs and are therefore useful in excluding sarcoidosis in our uveitis population. The biomarkers each separately have positive predictive values lower than 50%, which indicates their limitation in making a diagnosis of sarcoidosis. However, these PPV values are higher than the prevalence of sarcoidosis in our population and thus a positive test increases the suspicion of sarcoidosis. Clinically, this might be helpful for selecting patients in whom further investigations are justified such as a CT scan. The best way (so far) to raise a suspicion of sarcoidosis in uveitis population is to perform a chest radiography as this test has the highest PPV, but the PPV of CT scan and PET scan (which might be even higher) are unknown.

Ocular risk factors and the prevalence of systemic sarcoidosis

Inconclusive data exists on links between organ-specific sarcoidosis and progression to systemic disease.[23-31] In practice, the IWOS criteria showed high diagnostic performance in Japan (with high ocular sarcoidosis prevalence), but the usefulness of ocular characteristics was not confirmed in a study performed in the US.[32-34] More validation studies are needed to prove the utility of the ocular risk factors associated with systemic sarcoidosis. The work ahead is to identify a subset of uveitis patients (i.e. risk factors associated to systemic sarcoidosis), in which the prevalence of extraocular sarcoidosis will be higher. Existing diagnostic tests would perform better in a such circumstances.

Timeframe of development of extraocular sarcoidosis

A substantial proportion (44%) of patients with sarcoidosis-associated uveitis (without initial extraocular involvement) develop extraocular involvement within 12 months. However, the work presented in this thesis was performed mostly in patients with recent onset of uveitis. Notably, the proportion of sarcoidosis-associated uveitis patients without (not yet detectable) systemic sarcoidosis manifestations at uveitis onset, might be missed with screening by chest X-ray.[35] Pulmonary sarcoidosis is the most common extraocular finding at uveitis onset and manifests earlier than other organ systems.[35] The most rational way to follow patients with no apparent extraocular involvement, would be to repeat the diagnostic examinations after a considerable interval in time. Our findings on the diagnostic tests for sarcoidosis do not change the Dutch uveitis guideline, but information extracted from the investigations on sIL-2R and lymphopenia for the diagnosis of sarcoidosis might be added.

QFT-G testing in uveitis patients

The excess of uveitis without an established cause in the QFT-G positive patients suggests a genuine link between a (prior) infection with *M. tuberculosis* and the development of uveitis, as has been described before.[36] However, we found that one in every fourth QFT-G positive uveitis patients showed some form of pulmonary lymphadenopathy, without exhibiting any constitutional symptoms. Positive *M. tuberculosis* cultures in pulmonary lymph nodes were demonstrated earlier in sporadic (otherwise healthy) QFT-G positive uveitis patients.[37,38] Positive PCR analysis for *M.Tuberculosis* was also reported in QFT-G positive patients with uveitis without any constitutional symptoms.[39] This indicates that uveitis associated to active TB disease may be present in patients without constitutional symptoms or other evidence of active TB disease.

Profiling of expression of genes in the IFN pathway in peripheral blood of QFT-G positive patients with uveitis of unknown origin and no constitutional symptoms distinguished 3 different groups: 1. A profile resembling those with active pulmonary TB, 2. A profile resembling that of healthy controls and 3. Patients displaying an in-between gene expression pattern.[39] These data suggest a presence of ongoing low-grade infection with *M.tuberculosis* in (a part of) QFT-G positive patients with uveitis. If this is correct, dual categorization into latent and active TB is therefore not satisfactory, as there is probably a spectrum of infection activity in QFT-G positive patients. Another explanation of pathogenesis in TB-associated uveitis might be a hypersensitivity reaction to TB antigens and subsequent cross-reaction with ocular antigens, though the evidence of such a process is lacking up to this point.[40]

Diagnosis of TB-associated uveitis from ocular tissues is difficult even in patients with active systemic TB infection, as the ocular infection is paucibacillary and demonstration of bacilli in ocular fluid and/ or biopsy from ocular tissues is still problematic.[41,42] QFT-G is an indirect diagnostic test for TB, but does not distinguish an active infection from a latent one.

Therefore, at present, the combination of a compatible form of uveitis and a QFT-G test result is being interpreted as a presumptive diagnosis for TB-associated uveitis.[43] Findings of this thesis however, indicate that in a non-endemic country, only a minority of these patients show signs typical of ocular TB (such as serpiginous-like choroiditis and occlusive vasculitis). Our findings imply that the association of positive QFT-G test and uveitis might also be coincidental and additional tests are needed to better select patients in whom uveitis is really triggered by a prior (or ongoing but mild) TB infection. So far, in QFT-positive uveitis patients without systemic symptoms, only a treatment trial might distinguish a true TB-associated uveitis from the coincident finding of TB infection in a uveitis patient.

Viral uveitis

Viral anterior uveitis was since long commonly associated with HSV or VZV infections, especially in Western countries. With advanced diagnostic testing throughout time, other viral agents were discovered in patients with anterior uveitis. For example, Rubella virus was discovered as being associated with FUS in Western countries.[44] Many clinicians assumed that intraocular inflammation caused by RV presents always as FUS. In contrast, we show that RV-associated uveitis has a much wider range of manifestations. The presence of vitritis is commonly prominent but also a misleading sign as ophthalmologists do not consider RV-associated uveitis in the differential diagnosis of vitritis and tend to classify these patients as idiopathic intermediate uveitis. This possibly causes a significant delay of diagnosis. Findings of this thesis showed that every second patient with RV-associated uveitis had glaucoma and/or elevated intraocular pressure, which is considerably higher compared to a general uveitis population.[45-50] Treating uveitis specialists should therefore be aware of the higher risk of glaucoma in these patients when prescribing topical corticosteroid therapy, especially since these patients do not respond well to topical corticosteroids.

As glaucoma was the most common cause of permanent visual loss, discovering predictive factors will help identify patients at risk for developing glaucoma earlier in the course of disease. The association of RV PCR positivity and development of glaucoma should be evaluated in larger prospective studies. We recommend careful long term controls for intraocular pressure in patients with RV-associated uveitis.

We found that none of our patients with RV-associated uveitis was vaccinated at young age. This strongly suggests that RV-associated uveitis represents a late manifestation of postnatally acquired infection. One should keep in mind that life-long immunity against the RV does not develop in all vaccinated individuals.[51] This might lead to RV infection at later age, possibly characterized by different symptoms, including ocular involvement.

Infection with EBV is almost universal as a large proportion of the adult population is seropositive for this virus. The mere presence of EBV genome in intraocular fluid does not prove an association between this infectious agent and uveitis since EBV infected lymphocytes may persist indefinitely in the human body and can migrate into the eye. We found low prevalence of positive EBV PCR in intraocular fluid in a large uveitis series. When the EBV was documented by GWC, usually other infectious agents were also involved, which indicates that EBV might only be an innocent bystander. Moreover, most EBV GWC were very low and one additional dilution step would result in a negative GWC value. However, genuine EBV-driven uveitis might develop in immunocompromised patients as immune reactions to seemingly ‘innocent’ infectious agents are in these patients suppressed. In addition, EBV-driven lymphomas might develop in immunosuppressed patients.[52-54]

In summary, we evaluated various diagnostic methods used in daily uveitis practice for the initial work-up and the burden associated to this work-up. Uveitis patients have favorable visual prognosis at the cost of frequent visits to an ophthalmologist and intensive treatment in their first year after referral. Temporary visual loss is a underestimated burden of uveitis, however. Commonly used ACE and chest radiography remain an important mainstay for the uveitis practice whereas sIL-2R performs slightly better in excluding sarcoidosis compared to serum ACE. Surprisingly, lymphopenia was found to be a predictor of sarcoidosis. The additive value of these individual tests is not yet known. Inflammation markers such as ESR and/ or CRP do not have high diagnostic value in the work-up but in patients with unexplained uveitis and no established cause of very high ESR, HIV infection might be considered. The diagnosis of TB-associated uveitis is mainly presumptive, and our investigation shows that also patients with nonspecific uveitis and positive QFT-G testing might have (latent) TB-associated uveitis. EBV-associated uveitis remains an enigma, but if present, has a favorable visual prognosis and EBV testing in intraocular fluids is certainly not recommended for the initial intraocular fluid analysis. RV however, is one of the most common detectable viral agents causing uveitis and should be also suspected in patients with (unilateral) intermediate uveitis, as in this anatomical uveitis entity the diagnosis of RV is commonly overlooked. Surprisingly, PCR positivity for RV seemed a prognostic factor for development of glaucoma, the most common cause of severe visual loss in these patients, finding which might be related to high viral loads and possibly more tissue damage. We evaluated various methods used in daily uveitis practice to diagnose specific entities according to the Dutch uveitis guideline. Implementation of these findings in clinical practice might improve and speed up a correct diagnosis in patients with uveitis. Still, there are many unresolved questions.

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chapter 7.2

Samenvatting

Samenvatting

Dit proefschrift omvat een aantal onderzoeken naar de diagnostische waarde van diverse onderzoeken bij uveïtis patiënten. Daarnaast worden oogheelkundige afwijkingen van een aantal ziektebeelden specifiek omschreven.

Uveïtis is een verzamelnaam voor allerlei inwendige oogontstekingen. Er zijn verschillende oorzaken voor uveïtis. Uveïtis komt voor bij een aantal ziekten waarbij het afweersysteem verstoord is, zoals sarcoïdose, verschillende soorten reuma en multipele sclerose. Deze groep wordt ook wel systeemziekte genoemd, omdat de ziekte zich niet alleen tot het oog beperkt. Ook infecties kunnen een uveïtis veroorzaken (bacterie, virus of schimmel). De oorzaak wordt in ongeveer de helft van de patiënten echter niet gevonden. Om een systeemziekte op te sporen is onderzoek nodig dat kan bestaan uit bloedonderzoek en röntgenfoto's. Soms wordt ook oogvocht onderzocht. Er bestaan vier soorten uveïtiden: uveïtis anterior (aan de voorkant van het oog, waarbij het regenboogvlies ontstoken is), uveïtis intermedia (middenin, waarbij vooral het glasvocht ontstoken is), uveïtis posterior (aan de achterkant, waarbij met name vaatvlies en netvlies betrokken zijn) of panuveïtis (ontsteking in het hele oog). De soort uveïtis bepaald welk soort onderzoek moet worden aangevraagd, vastgelegd in de Nederlandse Uveïtis Richtlijn. De waarde van verschillende diagnostische testen in het aantonen van een oorzaak is momenteel echter onbekend.

Hoofdstuk 2 geeft een overzicht van de oogheelkundige prognose van uveïtis patiënten. Een goede gezichtsscherpte wordt uiteindelijk vaak bereikt in uveïtis patiënten, echter ervaren patiënten vaak een tijdelijke daling in gezichtsscherpte, gerelateerd aan de activiteit van hun uveitis. Bijkomende oogziekten komen vaak voor, zoals staar en een verhoogde oogboldruk. De patiënten worden hiervoor intensief behandeld middels diverse medicamenteuze behandelingen en operaties en bezoeken het ziekenhuis hiervoor gemiddeld 11 keer per jaar. Het traject dat een uveïtis patiënt doorloopt vooraf aan het bereiken van een goede gezichtsscherpte, is intensief en belastend.

In **hoofdstuk 3.1** wordt een overzicht gegeven van de kennis op het gebied van oogheelkundige sarcoïdose. De huidige inzichten betreffende oogafwijkingen, frequentie van oogbetrokkenheid, diagnostiek en behandeling worden uiteengezet. Sarcoïdose is een systeemziekte waarbij elk orgaan aangedaan kan zijn en ook een belangrijke oorzaak van uveïtis. Vaak manifesteert sarcoïdose zich tegelijkertijd in de longen en ogen. De longsarcoïdose kan worden vastgesteld middels een röntgenfoto van de borstkas. Bij een op de vijf sarcoïdose patiënten is betrokkenheid van het oog het eerste symptoom. Lokale behandeling is de eerste stap (met corticosteroïd druppels of injecties). Bij een visus-bedreigende ziekte of betrokkenheid van de oogzenuw is systemische therapie geïndiceerd

d.m.v. tabletten, injecties of infusen, waarbij gehele lichaam mee behandeld wordt en niet slechts de ogen. Betrokkenheid van de oogzenuw is zeldzaam, maar resulteert vaak in een slechte gezichtsscherpte. De traanklier en het bindvlies zijn ook vaak aangedaan, maar hebben als symptoom vaak slechts droge ogen.

Uveïtis is de meest voorkomende uiting van oogheelkundige sarcoïdose. De diagnose 'Oogheelkundige Sarcoïdose' is grotendeels gebaseerd op de criteria van de 'International Workshop on Ocular Sarcoidosis' (IWOS). Onder de criteria voor oogheelkundige sarcoïdose vallen onder andere ook een afwijkende röntgenfoto van de borstkas en een verhoogde waarde van de stof Angiotensine Converterend Enzym (ACE). De waarde van deze diagnostische criteria is echter bij uveïtis patiënten nog onduidelijk en moet grootschalig beoordeeld worden.

In **hoofdstuk 3.2, 3.3 en 3.4** worden de resultaten gepresenteerd van onderzoeken voor het aantonen van sarcoïdose bij uveïtis patiënten. **Hoofdstuk 3.2** beschrijft het onderzoek naar de diagnostische waarde van een röntgenfoto van de borstkas bij uveïtis patiënten. In dit onderzoek werd bij 15% van de onderzochte uveïtis patiënten een afwijkende röntgenfoto van de borstkas gevonden, meestal duidend op sarcoïdose. Patiënten met sarcoïdose hebben vaak een afwijkende longfoto maar ook een verhoogde ACE waarde in het bloed. Indien deze twee onderzoeken gecombineerd worden, worden nog meer sarcoïdose patiënten in de uveïtis populatie correct gedetecteerd. Daarom is de combinatie van de rontgenfoto van de borstkas en ACE bepaling in het bloed een goede manier om sarcoïdose aan te tonen. Echter zijn er ook sarcoïdose patiënten met een normale röntgenfoto zonder verhoogde ACE waarde. Met andere woorden is er zeker ruimte om betere diagnostische testen/ technieken te ontwikkelen, zodat elke uveïtis patiënt met sarcoïdose opgespoord kan worden.

In **hoofdstuk 3.3** hebben we twee soorten bloedtesten met elkaar vergeleken, de soluble Interleukine-2 Receptor (sIL-2R) en de ACE waarde. De sIL-2R is een bloedwaarde die een indicatie kan geven van de sarcoïdose activiteit, echter de waarde in het diagnosticeren van oogheelkundige sarcoïdose was onbekend. Wij hebben een groot aantal nog niet behandelde patiënten met uveïtis onderzocht. De sIL-2R bleek met name verhoogd te zijn bij sarcoïdose patiënten, maar soms ook bij HLA-B27 geassocieerde uveïtis en Varicella Zoster Virus (VZV)- geassocieerde uveïtis. De C-statistiek (een statistische toets die de kans beschrijft dat een diagnostische test aangedane individuen correct identificeert) was vergelijkbaar voor sIL-2R en ACE. De sIL-2R waarde in het bloed was echter iets gevoeliger dan de ACE waarde om sarcoïdose aan te tonen. Sarcoïdose patiënten hadden vaak zowel een verhoogde sIL-2R waarde als tegelijkertijd een afwijkende röntgenfoto van de borstkas. Onze resultaten tonen aan dat een verhoogde sIL-2R waarde en ACE vergelijkbare diagnostische waarde hebben in het vaststellen van sarcoïdose bij uveïtis patiënten.

In **hoofdstuk 3.4** onderzoeken wij een bepaald type bloedcellen (lymfocyten) in uveïtis patiënten. In een aantal eerdere onderzoeken werd een verband gevonden tussen een verlaagd aantal lymfocyten (lymfopenie) en sarcoïdose. Het voorkomen en de diagnostische waarde van deze lymfopenie bij uveïtis patiënten was echter onduidelijk. Resultaten uit onze studie laten zien dat lymfopenie ook bij uveïtis patiënten sterk gerelateerd is aan sarcoïdose. Patiënten met een lymfopenie hadden een 12 keer hogere kans op het hebben van sarcoïdose ten opzichte van uveïtis patiënten zonder sarcoïdose. De kans dat iemand geen sarcoïdose heeft als er een normaal gehalte aan witte bloedcellen is (de negatief voorspellende waarde; NVW) blijkt 94%. De afwezigheid van lymfopenie is daarom waardevol in het uitsluiten van sarcoïdose bij uveïtis patiënten. Deze NVW is vergelijkbaar met die van een röntgenfoto van de borstkas (95%) en sIL-2R (95%) uit eerdere onderzoeken, en is zelfs hoger dan die van ACE (87%). Tuberculose is een chronische bacteriële infectie die uveïtis kan veroorzaken. Bepaalde vormen van uveïtis posterior worden specifiek geassocieerd met tuberculose zoals de serpingeuze choroiditis en occlusieve vasculitis. Een actieve tuberculose infectie wordt bevestigd middels het aantonen van delende tuberculose bacteriën (een positieve tuberculose kweek). Bij tuberculose in de ogen is een dergelijke kweek moeilijk te bemachtigen omdat al een enkele bacterie uveïtis kan veroorzaken en ook omdat een biopt van het oog geen aantrekkelijk onderzoek is. De combinatie van een oogheelkundig beeld passend bij tuberculose met een positieve QFT-G test wordt vaak gebruikt als criteria voor een veronderstelde tuberculose-geassocieerde uveïtis, als tenminste alle andere oorzaken van uveïtis uitgesloten zijn.

In **hoofdstuk 4** onderzoeken we de toegevoegde waarde van het standaard uitvoeren van de QuantiFERON-Gold (QFT-G) test bij elke uveïtis patiënt. Dit is een bloed onderzoek dat de immuunreactie (de productie van de stof γ-interferon door witte bloedcellen) op tuberculose bacteriën meet. Een verhoogde waarde geeft aan dat de patiënt ooit besmet is geweest met de tuberculose bacterie.

Voor dit onderzoek zijn alle patiënten sinds de invoering van deze test in ons centrum geïncludeerd. Het bleek dat ongeveer een op de tien uveïtis patiënten positief is voor deze test. Bij een groot deel van de positief geteste patiënten bleek eigenlijk geen oorzaak voor uveïtis. Deze QFT-G positieve uveïtis patiënten, vertoonden geen specifieke oogheelkundige kenmerken, met name de beelden die eerdere met tuberculose geassocieerd waren zoals serpigineuze choroiditis of occlusieve vasculitis kwamen zelden voor. Dat is makkelijk te verklaren door de verschillende samenstelling van de onderzochte uveitis populaties. De oorzaak van uveïtis in deze patiënten ligt mogelijk in de (eerdere) infectie met tuberculose, maar kan ook op een toeval berusten. De kans op een toevallige associatie van uveitis en positieve QFT-G is natuurlijk hoger in de landen waar tuberculose vaak voorkomt.

Het uitvoeren van de QFT-G test is zeer nuttig in Nederland, met name omdat men in patiënten met een bedreiging van de gezichtsscherpte een proefbehandeling tegen de tuberculose bacterie kan rechtvaardigen. Verder onderzoek is nodig om betere testen te ontwikkelen die tuberculose-geassocieerde uveïtis patiënten goed onderscheiden van een uveïtis door andere oorzaak.

Hoofdstuk 5.1 is een algemene introductie tot (virale) uveïtis anterior (UA). Een virale oorzaak van UA wordt vaak gekenmerkt door een eenzijdige ontsteking van het voorste oogsegment. Daarbij kunnen zich kleine of middelgrote afzettingen van ontstekingscellen op het hoornvlies vormen (keratische precipitaten; KP's). Een verdunning van het regenboogvlies (irisatrofie), een verhoogde oogboldruk en vroege ontwikkeling van staar kunnen ook een teken zijn van virale UA. Een opvallende ontsteking van het glasvocht (vitritis) wordt vooral gezien zien bij rubella virus (RV)-geassocieerd uveïtis en Human T-Lymphotropic-Virus type 1 (HTLV-1)-geassocieerde uveïtis. De afwezigheid van synechiae (verklevingen van de iris met de lens) zijn kenmerkend voor RV-geassocieerde uveïtis, terwijl deze bij een VZV en Herpes Simplex Virus (HSV)-geassocieerde UA juist vaak voorkomen. Daarnaast presenteert VZV-geassocieerde UA zich vaker op oudere leeftijd met typerende huidblaasjes en is segmentale irisatrofie kenmerkend voor de HSV- en VZV geassocieerde UA. Een onderscheidend kenmerk van de cytomegalovirus (CMV)-geassocieerde UA is de corneale endotheliitis met munt-achtige KPs en een milde UA, vaak gepaard gaand met een hoog oogboldruk. De diagnose van een virale UA kan bevestigd worden door analyse van intra-oculair vocht middels polymerase chain reaction (PCR) of bepaling van de intra-oculaire antilichaam productie van het desbetreffende virus (Goldmann-Witmer Coefficient; GWC). De typerende klinische kenmerken beschreven in onze studie, kunnen gebruikt worden om een verschil te maken tussen specifieke virussen in omstandigheden waar een analyse van intra-oculaire vocht niet mogelijk is.

In **hoofdstuk 5.2 en 5.3** worden specifiek twee virale oorzaken van uveïtis besproken: de RV-geassocieerde uveïtis en de EBV-geassocieerde uveïtis.

In **hoofdstuk 5.2** onderzoeken we het spectrum van oogheelkundige afwijkingen en de meest voorkomende complicaties van RV-geassocieerde uveïtis. Een RV-geassocieerde uveïtis blijkt meestal eenzijdig te zijn en vaak was zowel het voorste oogsegment als ook het glasvocht aangedaan. Kenmerkend was dat deze patiënten nooit verklevingen hadden van het regenboogvlies met de ooglens (synechiae posterior). De ontsteking van het glasvocht (vitritis) was een van de frequentste klinische kenmerken (89%). De juiste diagnose van RV-geassocieerde uveitis werd vaak te laat gesteld, waarschijnlijk omdat de oogartsen in de aanwezigheid van vitritis niet aan het RV dachten. Een groot

deel van de patiënten ontwikkelde staar, een verhoogde oogboldruk en/ of glaucoom. Nauwe controle van de oogdruk in deze patiënten is daarom belangrijk voor de zorg op lange termijn.

Vaak wordt door oogartsen aangenomen dat het RV zich oogheelkundig presenteert met het Fuchs Uveïtis Syndroom (FUS; een constellatie van volgende oogheelkundige kenmerken: chronische uveïtis, iris atrofie, KP's, afwezigheid van synechiae posterior en aanwezigheid van staar). Wij vonden echter maar een klein deel van de patiënten met RV-geassocieerde uveïtis, die zich presenteerde als FUS (29%). De patiënten die zich met FUS presenteerden, waren wel bijna altijd positief voor het RV (95%). Ons onderzoek laat duidelijk zien dat vele gevallen van het FUS door het RV worden veroorzaakt, echter de RV-geassocieerde uveïtis zich niet per se presenteert als een FUS en een veel breder spectrum van klinische kenmerken omvat. Ook bij de patiënten met vitritis als voornaamste kenmerk dient gedacht te worden aan de mogelijkheid van RV geassocieerd uveitis.

Het blijft een raadsel of het Epstein-Barr Virus (EBV) daadwerkelijk uveïtis kan veroorzaken. In **hoofdstuk 5.3** beschrijven we de oogheelkundige karakteristieken van een groot aantal uveïtis patiënten die getest werden op de aanwezigheid van EBV in oogvocht. Slechts een klein deel is positief voor dit virus, terwijl dan tegelijkertijd vaak een alternatieve uveïtis oorzaak wordt gevonden en/ of EBV tegelijkertijd samen met andere virussen gevonden was. Een hele kleine groep van de geteste patiënten hadden alleen het EBV als mogelijke verklaring voor de uveïtis. Deze patiënten hadden vaak een aspecifieke uveïtis met een goede gezichtsscherpte. Wij concluderen dat een initiële analyse naar EBV bij uveïtis patiënten in het oogvocht niet nuttig is.

In **hoofdstuk 6** bepalen we de diagnostische waarde van twee aspecifieke ontstekingswaarden bij nieuwe uveïtis patiënten: de bezinkingsnelheid van erythrocyten (BSE) en c-reactief proteïne (CRP). De BSE is een waarde die verhoogd is als er meer eiwitten in het bloed te meten zijn, zoals bij een ontsteking. Het CRP is een acute fase eiwit, dat wordt losgelaten uit beschadigd weefsel. Allerlei soorten ontstekingen kunnen weefsel beschadigen en dit eiwit uitstoten. Een verhoogde BSE- en/ of CRP-waarde duiden daarom op een ontsteking ergens in het lichaam. Uit onze studie bleek dat een groot deel van infectieuze uveïtis eigenlijk normale BSE en CRP-waarden hebben (45%). Dit wordt waarschijnlijk veroorzaakt door het feit dat de infectie lokaal is en beperkt is tot het oog. Normale waarden bij een infectieuze uveïtis werden met name bij *Toxoplasma gondii* infecties gevonden (82%), een parasiet die bij een uveïtis slechts intra-oculair aanwezig is en zelden met een actieve systemische infectie gepaard gaat. Verhoogde waarden werden met name bij systemische infecties geobserveerd, zoals endogene endophthalmitis (71%).We hebben geen verband gevonden tussen verhoogde BSE- en/ of CRP-waarden en een specifieke oorzaak van uveïtis. Hierdoor

hebben verhoogde BSE- en/ of CRP-waarden niet een onderscheidend vermogen voor de uveïtis oorzaak, echter het toont aan of de ontsteking wel of niet systemisch aanwezig is.

Samengevat evalueerden we verschillende onderzoeken die gebruikt worden voor de diagnostiek van uveïtis en bekeken we oogheelkundige afwijkingen die kenmerkend zijn voor verschillende oogziekten. De ACE waarde en röntgenfoto van de borstkas zullen een belangrijke steunpilaar blijven in de diagnostiek van sarcoïdose-geassocieerde uveïtis. Daarbij is de sIL-2R waarde in het bloed iets gevoeliger dan de ACE waarde om sarcoïdose aan te tonen. Lymfopenie bleek verrassenderwijs ook een goede voorspeller van sarcoïdose te zijn in uveïtis patiënten. Toekomstig onderzoek is nodig om de waarde van een combinatie van deze testen te bepalen. De diagnose tuberculose-geassocieerde uveïtis is vaak een veronderstelde diagnose. Een positieve QFT-G test heeft eigenlijk geen diagnostische betekenis voor uveïtis patiënten, maar rechtvaardigt wel een behandeling tegen tuberculose in patiënten met bedreiging van de gezichtsscherpte. Aspecifieke ontstekingsmarkers zoals de BSE en CRP hebben geen diagnostische waarde voor uveïtis patiënten. De RV-geassocieerde uveitis heeft een veel breder spectrum van symptomen dan vroeger verondersteld en slechts een deel manifesteert met klassieke FUS beeld. De EBV-geassocieerde uveïtis blijft een raadsel, maar mits deze bestaat, komt het niet vaak voor en patiënten hebben een goede visuele prognose. Toepassing van de bevindingen uit dit proefschrift in de klinische praktijk zal een correcte diagnose in patiënten met uveïtis bespoedigen. Door het brede spectrum aan oorzaken van uveïtis, blijft het vinden van een oorzaak echter een uitdaging!

chapter 7.3

Özet

Özet

Bu tez çalışması üveit hastaları ile ilgili yapılan çeşitli çalışmaların teşhis değerlerini içeren bazı çalışmaları içermektedir. Üveit kelimesi tüm dahili göz enfeksiyonları için kullanılan bir terimdir. Üveit, sarkoidoz ve çoklu skleroz gibi bağışıklık sisteminin bozukluğu çeşitli hastalıklarda kendini gösterir. Bu gruba aynı zamanda sistemik hastalıklar da denir çünkü bu hastalık yalnızca göz hastalıkları ile sınırlı değildir. Enfeksiyonlar da üveit oluşturabilir (bakteri, virüs veya mantar). Ancak hastaların yaklaşık yarısında neden bulunmamıştır.

Sistemik bir hastalığı tespit etmek için kan testleri ve röntgen testlerini içeren bir muayene yapılması gereklidir. Bazı durumlarda oküler sıvılar da incelenir. Üveitin türü, Hollanda Üveit Yönergesi'nde belirtildiği gibi, ne tür bir muayenenin yapılması gerektiğini belirler. Fakat bazı tanı testlerinin değerleri bilinmemektedir.

2. Bölüm üveit hastalarındaki oftalmik prognoz ile ilgili genel bir bakış sunmaktadır. Nihayetinde iyi bir görsel keskinlik üveit hastalarında genellikle elde edilebilirken, ancak hastalar sıklıkla üveit hastalığının aktivitesine bağlı olarak geçici olarak görsel keskinlikte azalma deneyimleyebilirler. Katarakt ve yüksek göz tansiyonu da yaygındır. İyi bir görsel keskinliğe sahip olmadan önce hastaların geçirdiği süreç yoğun ve streslidir.

3.1 Bölüm Oküler sarkoidoz alanı ile ilgili genel bir bakış sunmaktadır. Sarkoidoz her türlü organı etkileyebilen bir sistemik hastalıktır ve üveitin asıl sebebidir. Sıklıkla sarkoidoz eş zamanlı olarak akciğerlerde ve gözlerde açığa çıkar. Akciğer sarkoidozu göğüs röntgeni ile tespit edilebilir. Lokal tedavi ilk basamaktır (kortikosteroid damlalar veya enjeksiyonlar ile birlikte). Sistemik terapi görsel beceriyi tehlikeye düşüren bir hastalık veya optik sinirlerin de hastalığa karışması durumlarında tablet, enjeksiyon veya direkt damara enfüzyon yoluyla uygulanır. Optik sinir, gözyaşı bezi ve konjunktiva da etkilenebilir. "Oküler sarkoidoz " teşhisi büyük oranda "Oküler Sarkoidoz Üzerine Uluslararası Çalıştay" (IWOS) kriterlerine bağlıdır (anormal göğüs röntgeni ve yüksek Anjiyotensin Dönüştüren Enzim (ACE) maddesi değerini de içerir).

3.2 Bölüm üveit hastalardaki göğüs röntgeninin tanı değerlerinin incelenmesini açıklar. Sarkoidozlu hastalar sıklıkla anormal akciğer röntgen değerlerine sahip olurlar ve ayrıca kandaki ACE değerleri yüksektir. Bu nedenle sarkoidozu tespit etmek için göğüs röntgeni ile kanda ACE testinin kombinasyonu iyi bir yoldur. Fakat yüksek ACE değerlerine sahip olmayan ve normal X-ray testi sonuçları alan sarkoidoz hastaları da vardır ve bu nedenle daha iyi teşhis testlerine ihtiyaç duyulur.

3.3 Bölümde çözülebilir Interleukin-2 Reseptörleri (sIL-2R) ile ACE değerlerini karşılaştırdık. sIL-2R sarkoidoz aktivitesini gösterebilen bir kan değeridir. Bu değer özellikle sarkoidoz teşhisi konulan hastalarda yüksek çıkmaktadır fakat üveit ile bağlantılı HLA-B27 ve üveit bağlantılı Varis Zoster Virüsü (VZV) hastalarında da bu değer yüksek çıkmaktadır. sIL-2R değeri üveit bağlantılı sarkoidoz teşhisi koyma konusunda ACE değerinden nispeten daha yararlıdır. Çalışma sonuçlarımız yüksek sIL-2R değerinin ve ACE değerinin üveit hastalarında sarkoidozun belirlenmesinde benzer tanı değerine sahip olduğunu gösteriyor.

3.4 Bölümde üveit hastalarındaki belirli bir kan hücresi türünü (lenfositler) inceledik. Lenfopeni rahatsızlığı olan hastalar sarkoidoz teşhisi konulmayan hastalara göre daha yüksek sarkoidoz riskine sahipler. Akyuvar hücrelerinin normal ölçüldüğü bir kişinin sarkoidoz olmama ihtimali (negatif öngörü değeri; NPV) %94'tür. Lenfopeni eksikliği bu nedenden dolayı sarkoidoz dışındaki üveit hastalarında önemlidir. Bu NPV ayrıca önceki çalışmalardaki göğüs röntgen testi (%95) ve sIL-2R (%95) değerleri ile karşılaştırılabilir, hatta ACE (%85) değerinden de yüksektir.

4. Bölümde QuantiFERON-Gold (QFT-G) testinin katma değerini analiz ettik. Bu testin yüksek değerde çıkması hastaya daha önceden tüberküloz bakterisi bulaştığını gösterir. Yaklaşık olarak her on üveit hastasından birinde bu test değerinin pozitif olduğu ortaya çıktı. Bu QFT-G pozitif değerli üveit hastaları daha önceden herhangi bir oftalmik belirti göstermemiştir, özellikle geçmişte tüberküloz rahatsızlığı ile ilgili resimler oldukça nadirdir. QFT-G testini uygulamak Hollanda'da oldukça kullanışlıdır çünkü görsel rahatsızlık riski taşıyan hastalar, tüberküloz bakterisine karşı bir test tedavisinin haklı olduğunu anlayabilir. Tüberküloz bağlantılı üveit tanısı için daha iyi testlerin geliştirilmesi için daha çok araştırma yapılması gerekmektedir.

5.1 Bölüm (viral) Ön üveit hastalığı (AU) hakkında genel bir giriş içerir. AU oluşum nedeni sıklıkla ön göz segmentinin tek taraflı iltihaplanması, korneada küçük veya orta büyüklükte iltihaplı hücrelerin yer alması (keratik presipitatlar; KP'ler), iris seyrelmesi (iris atrofisi), yüksek göz tansiyonu ve kataraktın başlangıç seviyeleri ile karakterize edilir. Posteriyor sineşileri VZV ve Herpes Simplex Virüs (HSV) bağlantılı AU'larda yaygındır. Ayrıca, VZV bağlantılı AU ileri yaşlarda kendini tipik cilt kesecikleri ile gösterirken segmental iris atrofisi HSV ve VZV bağlantılı AU'ların belirtilerindendir. Sitomegalovirüs (CMV) bağlantılı AU'larda bozuk para şeklindeki KP'ler ile birlikte kornea endoteliti ve yüksek göz tansiyonu önemli belirtilerdir. Viral AU tanısı polimeriz zincir reaksiyonu (PCR) ile intraoküler sıvının analizi sayesinde veya ilgili virüslerin intraoküler antibody üremelerinin (Goldmann-Witmer Katsayısı; GWC) tespiti sayesinde doğrulanabilir.

5.2 Bölümde RV bağlantılı üveit hastalığını inceledik. Genellikle bu hastalar daha önceden hiç posteriyor sineşisine yakalanmamışlardır. Vitröz iltihaplanması oldukça sıktır (%89). RV bağlantılı üveit hastalığının teşhisi doğru yapılmasında sıklıkla geç kalındı. Oftalmolojistler genellikle RV'nin kendini oftalmolojik olarak Fuchs Üveit Sendromu ile birlikte gösterdiğini kabul ederler (FUS; belirtilen oftalmik durumların birlikte görülmesi: kronik üveit hastalığı, iris atrofisi, KP'ler, posteriyor sineşi eksikliği ve katarakt rahatsızlığı). Fakat RV bağlantılı üveit hastalarının yalnızca küçük bir kısmında FUS olarak bulunduğunu (%29) tespit ettik. Çalışmamız pek çok FUS vakasının RV tarafından oluştuğunu açık bir şekilde gösteriyor fakat RV bağlantılı üveitler kendini FUS olarak göstermeyebilir ve çok daha geniş klinik özelliklere sahip olabilirler.

Epstein-Barr Virüs'ünün (EBV) gerçekten üveit oluşturup oluşturmadığı bir gizem olarak kalmaya devam ediyor. **5.3 Bölümde** bu virüsün üveit hastalarında yaygın bir şekilde görülmediğini gösterdik. Eğer test sonucu EBV için pozitif çıkarsa bu, üveit rahatsızlığının başka bir nedeni olduğunu gösterir ve bu gruptaki hastaların belirli bir oftalmik anormallikleri yoktur. Son olarak, üveit hastalarında oküler sıvının EBV için başlangıç analizinin kullanışlı olmadığını belirtiyoruz.

6. Bölümde üveit hastalarında iki nonspesifik iltihap değerini inceledik: eritrosit sedimentasyon oranı (ESR) ve c-reaktif protein (CRP). Yüksek ESR ve/veya CRP değerleri vücudun herhangi bir yerinde iltihap olduğunu gösterir. Çalışmamız bulaşıcı üveitlerin büyük bir kısmının normal ESR ve CRP değerine sahip olduğunu göstermiştir (%45). Bu büyük ihtimalle enfeksiyonun lokal bir olgu olmasından ve gözlerde sınırlı kalmasından kaynaklanmaktadır. Yüksek değerler özellikle iç kaynaklı endoftalmit (%71) gibi sistemik enfeksiyonlarda görülmektedir. Yüksek ESR ve/veya CRP değerleri üveit rahatsızlığı nedenlerini ayırt etmez fakat iltihabın sistemik olup olmadığını göstermektedir.

Özetle, üveit teşhisi için kullanılan çeşitli çalışmaları değerlendirdik. ACE değeri ve göğüs röntgen testi sarkoidoz bağlantılı üveit hastalığının teşhisi için önemlidir. Ayrıca kandaki sIL-2R değeri ACE değerine oranla sarkoidoz tespitinde daha hassastır. Limpofeninin beklenmedik bir şekilde üveit hastalarında sarkoidoz muayenesi için iyi bir belirti olduğu kanıtlanmıştır. Pozitif bir QFT-G testi üveit hastaları için tanısal bir öneme sahip değilken görsel keskinliğe karşı tehlike riskine sahip hastalarda tüberküloza karşı tedavi için önemli görülmektedir. ESR ve CRP gibi nonspesifik iltihap belirtileri ise üveit hastaları için herhangi bir tanısal öneme sahip değildir. RV bağlantılı üveit hastalığının önceden kabul edilenden daha geniş bir belirti kümesine sahip olduğu görülmüştür ve yalnızca bunun kısmı klasik FUS'u ortaya çıkarır. EBV bağlantılı üveit ise bir gizem olarak kalmaya devam ediyor. Fakat üveit hastalığına yol açan nedenlerin geniş spektrumlu olması bir nedenin bulunmasını zorlaştırmaktadır!

Epilogue

Dankwoord

Dankwoord

Promoveren doe je niet alleen, al heb ik vaak eenzame dagen achter de computer doorgebracht. Velen hebben eraan bijgedragen, direct en indirect, opdat dit boekje tot stand zou komen. Hier wil ik graag de ruimte geven aan allen, die dit traject mogelijk hebben gemaakt.

Prof. dr. A. Rothova, lieve Aniki. Bedankt voor je geduld tijdens mijn eigenwijze momenten. De momenten dat ik soms dacht alles beter te weten, maar er dan toch achter kwam dat een stap op de plaats te maken een betere zet was. Je hebt mijn, soms overenthousiaste, ideeën op een ontzettend leuke manier steeds in rechte banen weten te leiden. Tijdens dit proces heb ik altijd het gevoel gekregen dat we dit onderzoek echt samen uitvoerden. Daarboven uit wil ik je apart bedanken voor je altijd persoonlijke interesse en belang voor dingen in mijn leven buiten het ziekenhuis en je steun hierin!

Prof. dr. Vingerling, Hans, er zijn veel dingen waarvoor ik je erg dankbaar bent, de belangrijkste hiervan zijn de tussentijdse evaluaties. Het heeft me elke keer veel geleerd omdat je de onderzoeksprojecten steeds vanuit een epidemiologisch perspectief bekeek, elke bespreking was een echt leermoment voor mij, dat waardeer ik.

AbbVie Nederland wil ik hartelijk danken voor de financiering van dit promotietraject. Zonder jullie steun was dit boekje niet tot stand gekomen! Beste Rosanna, ik wil je persoonlijk bedanken voor je betrokkenheid in het verkrijgen van de financiering voor dit promotietraject.

Prof. dr. van Hagen, beste Martin. Ik heb bewondering voor jouw manier van lesgeven. Als student kreeg ik van jouw altijd het gevoel dat geen vraag dom is. Bedankt voor je interessante kritiek tijdens dit traject, ook tijdens de immunologie besprekingen.

Jan, nou had ik je, kort voor mijn promotie, bijna omver gereden bij de museumpark garage! Gelukkig heeft het de beoordeling niet negatief beïnvloed, allereerst wil ik je daarvoor bedanken ☺. Maar wat ik nog meer waardeer, was jouw heldere, kritische blik op de onderzoeksprojecten. Vooral je perspectief op de artikelen worden gewaardeerd, hoe ik de rode draad van een artikel goed naar voren moet brengen in een artikel, heb ik vooral met jouw eerlijke, korte en krachtige commentaar geleerd. Al valt er nog steeds veel te leren, natuurlijk!

Joke, ik wil je heel erg bedanken voor je begeleiding tijdens het Rubella project en de hulp bij het opzetten ervan. Het was niet mogelijk geweest zonder jouw en Suzanne! Je hebt een ontzettend fijne manier van opbouwende kritiek, welke ik altijd heel fijn heb gevonden. Josianne, ik kan oneindig over je collegialiteit gaan schrijven. Het eerste wat me te binnenschiet is, niet zeuren, maar poetsen! Dat is echt jouw mentaliteit en die waardeer ik zo!

Ik kan me geen enkel moment herinneren dat je ‘nee sorry, geen tijd’, tegen me hebt gezegd. Je staat altijd klaar voor je collega’s, voor een lachje, een koffie, maar het allerbelangrijkste, voor samenwerken. Heel veel succes met je scleritis project!

NOOIT ga ik het lab in, zei ik tegen Aniki aan het begin van mijn promotietraject. Toch is het ervan gekomen, maar zonder enige spijt. Het bleek zelfs leuk te zijn! Ik wil apart dank betuigen aan de mensen die dit een leuke ervaring hebben laten worden. Nicole Nagtzaam, dank voor je samenwerking en snelle resultaten. Bedankt, Wim Dik voor je interessante gesprekken over het sIL-2R project, de nauwe samenwerking en je betrokkenheid bij de tussenevaluaties. Met name dat laatste vond ik ontzettend fijn. Daarnaast wil ik ook Annemiek van der Eijk-Baltissen en Sandra Scherbeijn bedanken voor jullie fijne en snelle samenwerking op het Virologie lab.

Verder wil ik nog diegene bedanken die feedback gegeven hebben op presentaties over diverse onderzoeksprojecten (ik ken jullie niet allemaal ☺). Het zijn er teveel om op te noemen, zij die aanwezig waren op de lunchbesprekingen van de oogheelkundige afdeling van het Erasmus MC, de landelijke uveitisvergadering en het uveitisdagdeel van het NOG, dat elk jaar plaats vond. Ik heb het meeste geleerd door te presenteren en nog meer van de vragen die naar aanleiding hiervan werden gesteld.

In het bijzonder wil ik Sharon, onze physician assistant in opleiding, bedanken. Ik vind je een ontzettend fijne collega. Hard werkend en goed samenwerkend. Ik heb veel van je geleerd en wil je met name bedanken voor het feit dat ik altijd, maar dan ook altijd, bij je terecht kon met een vraag om hulp of een inhoudelijke vraag van het vak. Ik heb er vertrouwen in dat je en goede physician assistant zult worden en wens je heel veel succes met je opleiding. Ook wil ik Suzanne niet vergeten, wat hebben we gerubelleerd in Utrecht! Een project waar geen einde aan leek te komen, maar het samenwerken met jouw en Joke was me altijd een genoegen.

Laura, ik denk dat er een aparte dynamiek is ontstaan tussen ons tijdens onze gezamenlijke projecten. Ik kreeg altijd een overvloed aan ideeën en inspiraties door de gesprekken met jouw. Werken met jouw was altijd ontzettend prettig, helder en snel. Maar tussendoor ook nog tijd voor grappige momenten. Ik waardeer je inzet enorm en wens je ontzettend veel succes in je verdere carrière!

Buket, volledig op de achtergrond en onzichtbaar heb je me nooit afgewezen als ik een kritische blik nodig had over een artikel of abstract. Met name als ik door de bomen het bos niet meer zag, was dit erg fijn ☺. Ik wens je heel veel succes met je eigen promotietraject en sta ook altijd klaar voor jouw.

En dan wijlen mijn vader. Babacığım, wat jammer dat je dit moment en het traject vooraf gemist hebt, eyvallah. Ook al was je er lijfelijk niet bij, op sommige momenten heb ik steeds jouw voorbeeld van totale rust onder allerlei stressvolle omstandigheden voor me gehaald, en mezelf weten te kalmeren en motiveren uit gesprekken die we vroeger hebben gehad. Je inspirerende gesprekken van toen, hebben nog steeds effect vandaag. Bedankt voor je onvoorwaardelijke liefde en steun.

Atilla efendiciğim ve Hülya validem, sizlere ne kadar teşekkür etsek az. Dun ki gibi hatırlıyorum, sizi aramıştım bu doktora'yı yapmam için beni işe aldılar diye. Şimdi de sonucu elinizde efendiciğim. Allah razı olsun sizlerden ve tüm ihvanımızdan.

Lieve Mama, ik weet niet waar ik moet beginnen. Nadat ik zelf moeder ben geworden tijdens mijn promotietraject, begrijp ik steeds beter wat je eigenlijk allemaal hebt ingezet van jezelf, zodat ik kan staan waar ik nu sta. Het was niet mogelijk geweest, letterlijk en figuurlijk, zonder jouw. Ook voor je inzet met Hamza, je bent zo'n lieve oma, wil ik je extra bedanken. Zonder jouw zou dit boekje wat moeilijker tot stand gekomen zijn ☺.

Sevgili Sefa Babam'a, Melek validem'e, Sumeyye'me, Abdullah'a ayriyetten teşekkür borcum var. Bazen o hastenede ne yaptığımı anlatamıyordum sanki size. Sonucu şuan elinizde işte. Ve inanın, belki farkında olmadan bile, bu işimi de çok kolaylaştırdınız. Rabbim sizlerin de işlerinizi kolaylaştırsın. Ayrıca her daim gülümsemenizi esirgemediğiniz için ve canım oğluşum Hamza'ya cok güzel bir babaanne, dede, hala ve amca olduğunuz icin, sevginizi ve ilginizi ona verdiğiniz icin ayriyetten teşekkür borcum var. Dilerim ki sizin yüzünüzde her daim gülsün.

Hayat arkadaşım, goncam. Jij een eigen zaak, ik mijn promotie, en dan kwam Hamza er nog eens bij. Het is zo ongelooflijk druk geweest, maar jij hebt me altijd laten lachen in alle drukte en stress, tussen alles in. Geri kalan teşekkür borcum aramızda kalsın, biliyorsun, hakkını ödiyemem. Sonsuz desteğin için, ne kadar teşekkür etsem az. Rabbim seninde işlerini rast getirsin ve hayırlı işlerin olsun.

About The Author

About The Author

Fahriye Hakan-Groen was born on the 9th of August, 1988 in Berlin, Germany. She grew up in Berlin until her 11th year and continued here to secondary school in the Netherlands, Rotterdam. She graduated from secondary school in 2006 from Maarten Luther Christelijke Scholengemeenschap (Rotterdam, the Netherlands) and started her medical school in 2008 at the Erasmus Medical Center in Rotterdam, after studying one year of Pharmacy in Utrecht, the Netherlands. During her study she participated in a research master and obtained a Master in Health Sciences (NIHES) in 2014 under the supervision of prof. dr. A. Rothova. After receiving her medical degree she started a PhD project again under the supervision of prof. dr. A. Rothova. Fahriye presented her work at several national and international meetings. In June 2019 she will start her residency in Ophthalmology at the department of ophthalmology at the Erasmus Medical Center, headed by prof. dr. J.R. Vingerling.

Phd Portfolio

Phd Portfolio

Name PhD student:	F. Hakan-Groen
Erasmus MC Department:	Ophthalmology
PhD period:	November 2015-April 2019
Promotors:	Prof.dr. A. Rothova, Prof.dr. J.R. Vingerling

Presentations

2016 Uveitis workgroup: 'Chest X-ray screening for sarcoidosis in patients with active uveitis'

2017 Uveitis workgroup: 'sIL-2R and ACE in the diagnosis of sarcoidosis-associated uveitis'

2016 Department of Ophthalmology: 'Chest X-ray screening for sarcoidosis in patients with active uveitis'

2017 Department of Ophthalmology: 'Ocular sarcoidosis and ESR/CRP in uveitis patients'

2018 Department of Ophthalmology: 'Rubella virus-associated uveitis'

2018 Department of Ophthalmology, Utrecht: Rubella

(Inter)national conferences

2015 Dutch Ophthalmology PhD Students 3rd conference (DOPS), Nijmegen, The Netherlands

2016 Dutch Ophthalmology PhD Students 3rd conference (DOPS), Nijmegen, The Netherlands: 'Chest X-ray screening for sarcoidosis in patients with active uveitis' (Poster Presentation)

2017 Dutch Ophthalmology PhD Students 3rd conference (DOPS), Nijmegen, The Netherlands

2018 Dutch Ophthalmology PhD Students 3rd conference (DOPS), Nijmegen, The Netherlands: 'sIL-2R and ACE in the diagnosis of sarcoidosis-associated uveitis' (Oral Presentation)

2016 'Eilanddagen Oogheelkunde' Congress, Schiermonnikoog, The Netherlands

2017 'Eilanddagen Oogheelkunde' Congress, Schiermonnikoog, The Netherlands

2018 'Eilanddagen Oogheelkunde' Congress, Schiermonnikoog, The Netherlands

2017 Nederlands Oogheelkundig Gezelschap (NOG) jaarvergadering, Maastricht, The Netherlands: 'Erythrocyte sedimentation rate and C-reactive protein in patients with active uveitis' (Oral Presentation)

2018 Nederlands Oogheelkundig Gezelschap (NOG) jaarvergadering, Groningen, The Netherlands: 'Rubella Virus-associated uveitis' (Oral Presentation)

2016 9th International Symposium on Uveitis (ISU), Dublin, Ireland: 'Chest X-ray screening for sarcoidosis in patients with active uveitis' (Oral Presentation)

2019 IUSG/ICO 7the Venice Uveitis Course

Other academic activities

2015-2019	Weekly clinical work at uveitis outpatient department of ophthalmology, Erasmus MC
2015-2019	Uveitis workgroup meetings, Utrecht, The Netherlands
2015-2019	Weekly clinical ophthalmology-immunology seminars, Erasmus MC
2015-2019	2-weekly clinical ophthalmology patient seminars
2015-2019	weekly fluorescence angiogram seminars

List Of Publications

List Of Publications

Groen F, Ramdas W, de Hoog J, Vingerling JR, Rothova A.Visual outcomes and ocular morbidity of patients with uveitis referred to a tertiary center during first year of follow-up. Eye (Lond). 2016 Mar;30(3):473-80.

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