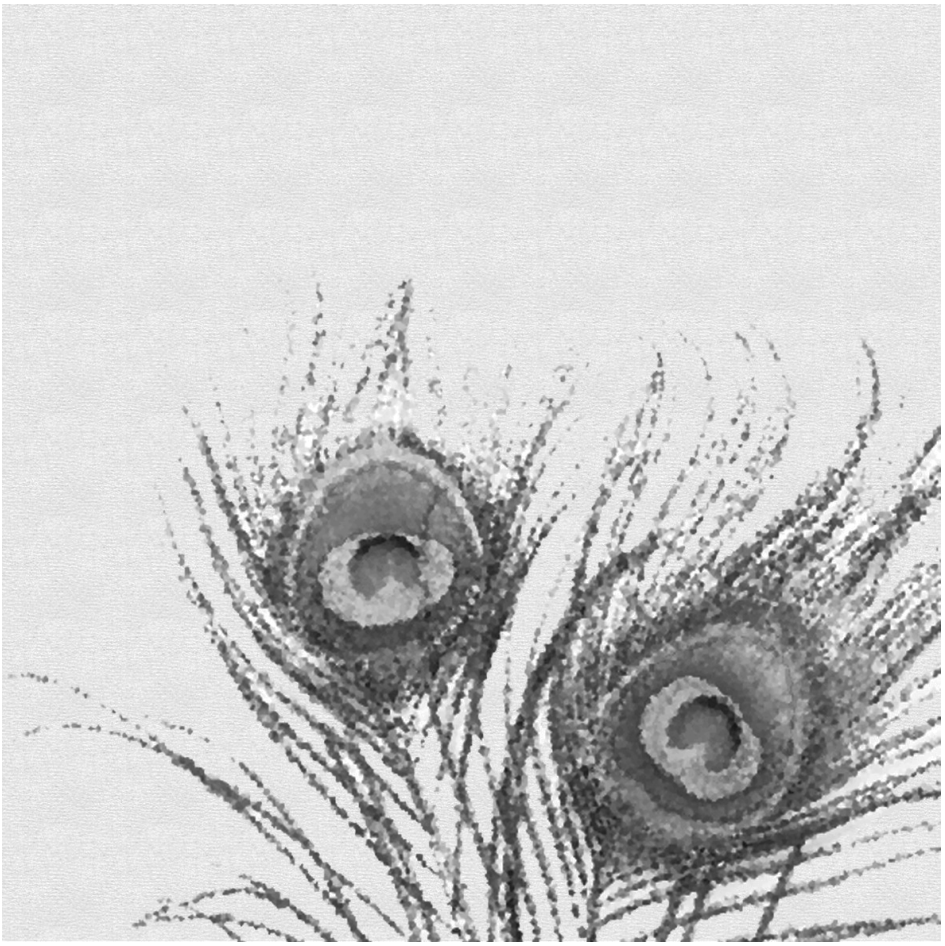
Uveitis in childhood

Clinical and fundamental developments



Viera Kalinina Ayuso

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Uveitis in childhood
Clinical and fundamental developments

Uveitis bij kinderen. Klinische en fundamentele ontwikkelingen.
(met een samenvatting in het Nederlands)

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in het openbaar te verdedigen
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Viera Kalinina Ayuso
geboren op 19 december 1980
te Moskou, Rusland

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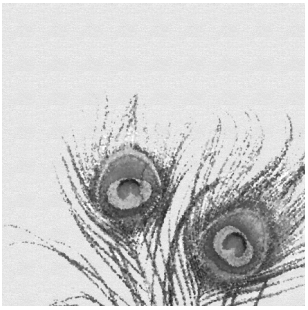
Ymkje Hettinga

Моим любимым родителям; за вашу безусловную любовь и поддержку

Aan mijn lieve ouders; voor jullie onvoorwaardelijke liefde en steun

Contents



Chapter 1

General introduction and aims of the study

General introduction

Epidemiology

Uveitis, inflammation of the middle vascular layer of the eye, is a disorder with many diverse causes and clinical appearances. It is relatively uncommon in children with the annual incidence rate of 4.3 - 6.9/ 100,000 and the prevalence of about 30/ 100,000.[1-3] Children with uveitis represent about 5% of the total uveitis patient population.[3;4]

Impact and visual prognosis

Despite its relatively low prevalence, uveitis in childhood is a serious blinding disease which represents a challenge for ophthalmologists due to its potential for long-term complications and visual disability. It is estimated that one-fourth to one-third of uveitis cases in childhood result in severe loss of vision.[5] Unilateral blindness occurs in 17%-24% of bilateral cases and in about 35% of unilateral pediatric uveitis cases. Unilaterally decreased vision lower than 20/40 can be found in 17-41% of the patients.[1;4;6] Due to frequently asymptomatic presentation the diagnosis is often delayed which can worsen the visual prognosis.[7]

Clinical patterns

Pediatric uveitis, similarly uveitis in adults, has diverse etiologies, and the systemic associations in children differ from those in adults. Uveitis in childhood is mostly of non-infectious origin, however in up to 60% of the cases underlying cause cannot be found.[2-4;6-8] Anterior uveitis is the most common anatomic type of uveitis in children; it accounts for 30 - 91% of the cases from referral centres all over the world.[2;8] Chronic bilateral anterior uveitis is typical for the most common systemic association of uveitis in children, namely Juvenile Idiopathic Arthritis (JIA) (Figure 1). Ophthalmologic screening is essential for early diagnosis of uveitis in JIA patients due to its usually insidious onset. In addition, chronic anterior uveitis may also precede arthritis or occur without systemic associations.[8;9]

The second most common uveitis entity in childhood is intermediate uveitis, which is characterised by a chronic inflammatory process primarily involving the vitreous, posterior part of ciliary body and peripheral retina (Figure 2).[10] This type of uveitis accounts for approximately 25% of childhood uveitis with outliers up to 42% in some referral centres.[2;8] This type of uveitis in children is usually idiopathic and frequently has initially an asymptomatic course.[2;11]

Posterior uveitis in children is relatively uncommon in children and like in adult pa-

tients, it is most frequently caused by toxoplasmosis.[1;3]

Pathogenesis

The pathogenesis of many non-infectious uveitis entities is still poorly understood, however uveitis is traditionally considered as an autoimmune disorder, which is caused by the loss of immune tolerance to autoantigens and subsequent activation of autoreactive T lymphocytes.[12-15] Insight into the intraocular immunological processes is mainly based on animal models, which have provided a valuable knowledge on the pathogenesis of uveitis in general.

Pathogenesis of JIA-associated uveitis is discussed in details in chapter 2 of this thesis. The current prevailing opinion is that JIA is a multifactorial genetically predisposed autoimmune disorder that can be influenced by environmental factors and infections. However, the specific pathogenesis of JIA-associated uveitis is not understood, nor has the basis of the relationship between the eye and joint inflammation been established.

Diagnosis

Early diagnosis of uveitis in children is very challenging due to several reasons. Firstly, as mentioned above, many pediatric uveitis entities are initially asymptomatic. Secondly, even in advanced cases children and their parents may be for a long time unaware of developing visual impairment. And thirdly, young children may be less cooperative for a complete ophthalmologic examination, so the diagnosis can be missed. Childhood uveitis is frequently idiopathic.[1;2] However, because many forms of uveitis can be a part of a more widespread inflammatory process it is essential to search for its systemic associations and exclude infections in a multi-disciplinary team including an ophthalmologist specialized in uveitis, a pediatrician with expertise in auto-immune disorders and a virologist/microbiologist. It is essential to exclude infectious causes of uveitis before starting anti-inflammatory and immunosuppressive treatment, which can be achieved with ocular fluid analysis for the most highly suspected infectious pathogens.

Complications

Because the diagnosis of uveitis in children is often delayed, severe complications may already be present at the moment when uveitis is finally detected.[6] These complications may result in loss of vision and can also cause amblyopia in young children.[2;4;5;7] The presence of complications at initial examination appears to be an important risk factor for development of new complications.[16]

The most frequent vision-threatening complications in pediatric uveitis in general are cataracts (35-52%; Figure 1), optic disc edema (29%; Figure 3), secondary glaucoma (19-33%; Figure 4) and cystoid macular edema (17-35%; Figure 5). However, many other anterior and posterior segment complications can worsen the course of uveitis in children and be the cause of visual disability as well.[4;6] Complications that are most frequently responsible for blindness in these patients include macular scars (27%), secondary glaucoma (15%), cystoid macular edema (8%), optic neuritis (8%), hypothony/atrophy (8%) and retinal detachment (8%).[4] Within various pediatric uveitis entities, JIA-associated uveitis has the highest complication rate.[2]

Management

Most entities of pediatric uveitis represent a chronic disease, and in consequence their management is often prolonged and complex. It can be very challenging and demanding for all involved parties: the physician, the patient and his/her family. The management of uveitis can be divided in anti-infectious, anti-inflammatory therapy and surgical treatment. The main aim of anti-inflammatory and immunosuppressive treatment is control of the inflammation and prevention of the development of sight-threatening complications with an acceptable level of side effects. Surgical treatment is essential in the management of frequently occurring complications.

Infectious uveitis is treated with antibiotic or antiviral drugs, sometimes in combination with anti-inflammatory drugs to limit the complications of the inflammatory process. In non-infectious uveitis, topical corticosteroids are the first step to control anterior segment inflammation. For intermediate and posterior uveitis, periocular or subtenon corticosteroid injections can be used as a first-line treatment option. Topical and periocular corticosteroid treatment is however frequently insufficient to achieve long term remission of ocular inflammation and its chronic use can enhance the development of cataract and secondary glaucoma. Therefore, systemic anti-inflammatory and immunosuppressive medication is warranted in many cases.[5;8;16;17]

Systemic corticosteroids are used mostly as a short-term option in children due to their numerous systemic side effects and the risk of cataract development and glaucoma. The dose of oral corticosteroids should be tapered as soon as possible, however, in case of recurrent inflammation additional steroid-sparing immunosuppressive medication should be considered. The choice of immunosuppressive drugs with most frequent prolonged administration in pediatric uveitis consists of antimetabolites (methotrexate, azathioprine and mycophenolate mofetil) and T cell inhibitors (cyclosporine). Alkylating agents (cyclophosphamide and chlorambucil) are only being used as a rescue

medication in emergency cases due to their potential for serious systemic side-effects. Methotrexate is frequently the agent of the first choice, especially in management of JIA-associated uveitis due to its effectiveness and general safety. Treatment with biologicals, monoclonal antibodies against specific elements of the immune system, is another alternative or additional treatment option in these patients. Adalimumab and infliximab are agents inhibiting tumor necrosis factor alpha (anti-TNFα) by binding its both soluble and transmembrane forms, they are being successfully used for the management of chronic pediatric uveitis, especially in JIA.[8] Another anti-TNFα agent, etanercept, a portion of the TNF receptor, which binds solely to the transmembrane form of TNFα is not effective in the treatment of uveitis.[8] Alternative biological agents in uveitis are anakinra, daclizumab, tocilizumab, abatacept, en rituximab, however the experience with their use in this population is very limited so far.

Development of certain sight-threatening complications such as uncontrolled secondary glaucoma and retinal detachment require urgent surgical treatment. While in other, less urgent, cases of secondary glaucoma or cases of cataract formation elective surgery on an eye without active inflammation can be performed. It is generally advisable to reach control of intraocular inflammation for at least 3 months prior to surgery if the ocular condition allows it.[16]

Trabeculectomy is a treatment option for secondary glaucoma; however, children with uveitis carry a high risk for trabeculectomy failure due to their predisposition to excessive fibrosis.[18] Glaucoma implant valve devices such as an Ahmed glaucoma valve have been proven effective for lowering intraocular pressure in such cases (Figure 6).[19]

Cataract surgery in children with uveitis can be challenging due to high rate of preexisting and eventual postoperative complications. Also implantation of intraocular lenses in these patients has for a long time been a subject of controversy due to potential development of serious postoperative complications such as hypotony, membrane formation and cystoid macular edema development.[16;20] However, specialized centers with expertise in modern surgical techniques and good perioperative control of inflammation report good visual results in pseudophakic eyes with pediatric uveitis.[21;22] Further, no difference in development of long-term complications between aphakic and pseudophakic eyes was noted while pseudophakic eyes had significantly better visual outcomes from 1 up to 7 years of post-surgical follow -up.[22]

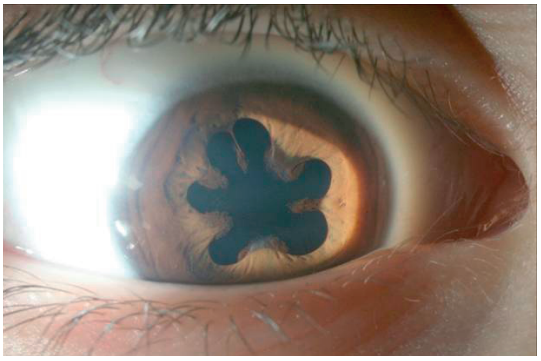


Figure 1 Slit-lamp photograph of chronic anterior uveitis, characterized by posterior synechiae

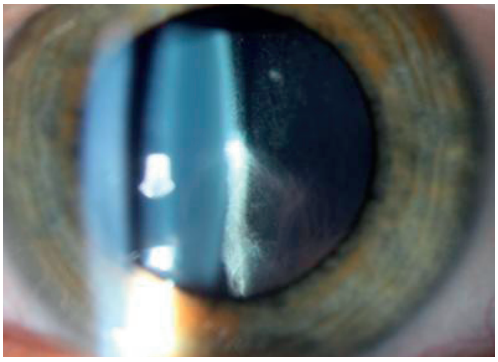


Figure 2 Slit-lamp photograph of intermediate uveitis, characterized by inflammatory cells and opacities in the vitreous

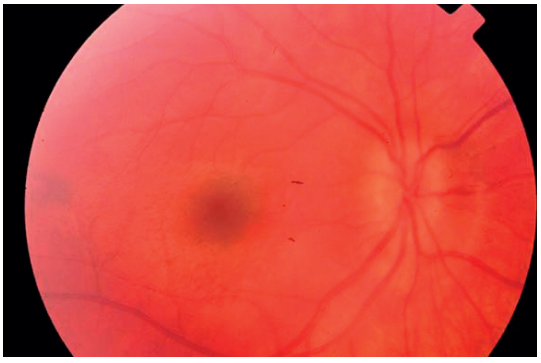


Figure 3 Fundus photograph shows optic disc edema

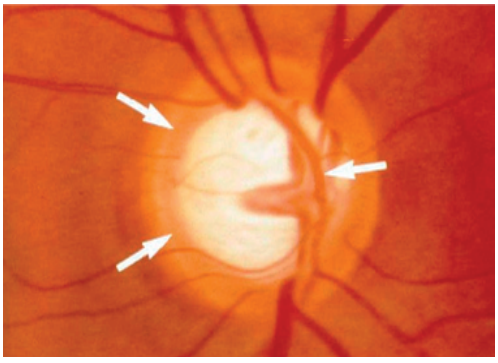


Figure 4 Fundus photograph shows advanced cupping of the optic disc in secondary glaucoma

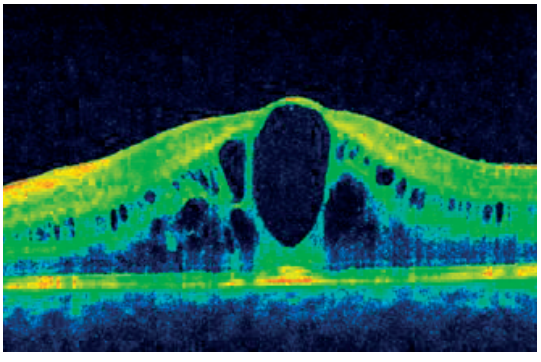


Figure 5 Optical coherence tomography shows cystoid macular edema.

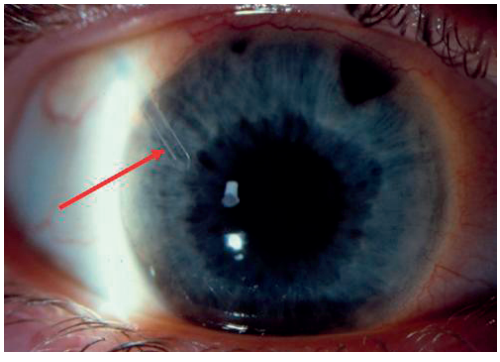


Figure 6 Slit-lamp photograph shows the tube of an Ahmed glaucoma valve implant in the anterior chamber of a patient with previous trabeculectomy.

Aims of the study

The general objective of this thesis was to gain new insights regarding the course and prognostic factors of uveitis in childhood, its pathogenesis of JIA-associated uveitis and the treatment of uveitis in children. Specific aims of this thesis are described below in four categories.

I. Uveitis course and prognostic factors

- The two most common uveitis entities in childhood are uveitis associated with JIA and intermediate uveitis. Both types of uveitis may follow a severe and a complicated course. Prognostic factors in these uveitis entities are not clearly identified and the results of the existing studies regarding the roles of age of onset and gender are often controversial. The purpose of our study was to evaluate the prognostic role of gender and age of onset of uveitis for a complicated course and visual outcomes in JIA-associated uveitis and intermediaire uveitis.
- There is lack of data on the long-term course of uveitis in JIA in different age periods. Additionally, there is no explanation for the fact that girls with JIA have a higher risk of developing uveitis compared to boys. Whether hormonal influences are involved is still unknown. We studied the influence of puberty on the course of uveitis progression over time and specifically determined the activity of uveitis, ocular complications and medical treatment in children with uveitis associated with JIA before and during puberty.
- Most children with JIA-associated uveitis are being diagnosed after the onset of arthritis. However, in some children, uveitis is the first sign of JIA with arthritis being diagnosed later. We wished to evaluate the percentage of these children and the interval till diagnosis of arthritis after the diagnosis of uveitis. Additionally, the prognostic role of this atypical manifestation of JIA for the uveitis course was of our specific interest.

II. Pathogenesis of JIA-associated uveitis

- Keeping in mind the atypical presentation of JIA with uveitis prior to arthritis and the absence of a definitive diagnostic test, there is a strong rationale for efforts to identify specific biomarkers in the ocular fluid of children with JIA-associated uveitis. We performed a proteomic analysis of paired aqueous humor and serum samples of children with different types of uveitis and non-inflammatory pediatric controls, attempting to identify intraocular JIA-specific biomarkers which could

provide new insights in its molecular mechanisms and perhaps find a potential clinical use in the future for diagnostic, prognostic or therapeutic purposes.

- Cellular infiltrate in JIA-uveitis is still poorly characterized. Almost all scarce histopathological studies of JIA-associated uveitis are performed on enucleated eyes and concern the end stage of the disease. In the present study we report on histopathological and immunohistochemical findings in a relatively large number of iris specimens obtained during therapeutic glaucoma surgery of children with various uveitis entities and pediatric non-inflammatory controls to characterize the composition of the inflammatory infiltrate in these eyes which would provide us with a better understanding of local immunologic processes in JIA-associated uveitis and other pediatric uveitis entities.

III. Treatment

- Topical corticosteroids are the first step of treatment in JIA-uveitis, but local therapy is frequently insufficient and chronic use of topical corticosteroid can enhance the development of cataract and glaucoma. Therefore, systemic immunosuppressive medication is warranted in many cases. Although methotrexate has been recognized as an effective treatment option for JIA-associated uveitis, there are still some unanswered questions about the optimal duration of methotrexate therapy and the risk of relapse of uveitis after discontinuation of methotrexate therapy. In this study we attempt to clarify these issues.
- Glaucoma is a common complication that can arise secondary to uveitis as well as to the use of local corticosteroid treatment. Children with uveitic glaucoma carry a high risk for trabeculectomy failure. Glaucoma implant valve devices such as an Ahmed glaucoma valve implant have been proven effective for lowering intraocular pressure in refractory glaucoma. However, their long-term effects on the corneal endothelium in inflammatory glaucoma in children are unknown. The purpose of the present study was to investigate the effect of an Ahmed glaucoma valve implant on corneal endothelial cells in children with glaucoma secondary to uveitis.

IV. Ocular complications and risk of uveitis in immunocompromised children after allogenic hematopoietic stem cell transplantation (HSCT)

- Uveitis in children is mainly of non-infectious origin. However, in immunosuppressed patients opportunistic infections may potentially affect the eye and cause uveitis. Allogenic hematopoietic stem cell transplantation (HSCT) has gradually gained a broad application in treatment of hematological malignancies as well as

immune deficiencies and metabolic diseases in children. Opportunistic infections in the first months after HSCT play a major role in the morbidity and the mortality of these patients, due to their immunosuppressed status. No systematic prospective studies have been performed to investigate the risk of ocular pathology in the pediatric HSCT population. Due to the improving clinical control of these infections and lower mortality, it is essential to have insights in the risk of ocular involvement following HSCT. We prospectively studied the ocular findings in children before and during the first year after an allogenic HSCT. We specifically focused on the eventual ocular involvement during systemic viral reactivations and investigated whether an ophthalmologic screening is warranted for pediatric HSCT population.

Outline of the thesis

Chapter 1 gives a general overview of uveitis in childhood and introduces the aims of the current thesis.
Chapter 2 provides a detailed review of the literature regarding the pathogenesis of JIA-associated uveitis.
Chapter 3 describes the analysis of the prognostic role of baseline factors on long-term development of ocular complications in JIA-associated uveitis by performing a retrospective analysis on 117 affected eyes of 65 patients with JIA-associated uveitis. Baseline factors which prognostic role we specifically analyzed include gender, age of onset of uveitis and initial manifestation of JIA (as initial arthritis with later uveitis or initial uveitis with later arthritis).
In **chapter 4** the same database of 117 eyes with JIA-associated uveitis was used to analyze visual outcome in JIA-associated uveitis according to gender, age of onset of uveitis and initial manifestation of JIA-associated uveitis (as initial arthritis with later uveitis versus initial uveitis with later arthritis).
Chapter 5 investigates the course and activity of JIA-associated uveitis in childhood and puberty by performing a retrospective analysis of clinical data of 62 patients with JIA-associated uveitis.
Chapter 6 contains the evaluation of the efficacy of methotrexate treatment and the effect of its withdrawal on relapse rate of JIA-associated uveitis by performing a retrospective analysis of data of 22 pediatric JIA patients who were being treated with methotrexate for active uveitis.
Chapter 7 describes the search of biomarkers in aqueous humor from patients with JIA-associated uveitis by performing a proteomic analysis using Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-ToF MS) of aqueous humor (n=73) and serum (n=105) samples from a total of 116 children including patients with JIA-associated uveitis, idiopathic uveitis clinically suspect for JIA association, other pediatric uveitis entities and non-inflammatory pediatric controls.
Chapter 8 characterizes cellular infiltration in iris specimens from 24 eyes with uveitis diagnosed before the age of 16 years and 6 eyes with open angle non-uveitic glaucoma. We evaluate the histopathological and immunohistochemical findings in JIA-associated uveitis and compare them to other pediatric uveitis entities and controls.
Chapter 9 identifies prognostic factors in intermediate uveitis in children by performing a retrospective data analysis of 35 patients with onset of intermediate uveitis before 16 years.

Chapter 10 reports on 3 otherwise healthy children with a co-existence of idiopathic intermediate uveitis and alopecia areata. The combination of these two conditions has not been previously described in the literature.

Chapter 11 evaluates the effect of Ahmed glaucoma valve implants on corneal endothelial cell density in children with uveitic glaucoma by performing a cross-sectional analysis of endothelial cell densities on 80 eyes from 42 patients with pediatric uveitis. Twenty-eight eyes had an Ahmed glaucoma valve implant as treatment for secondary glaucoma. Fifty-two eyes without an implant served as controls. We correlate endothealial cell densities in these eyes with the presence of an Ahmed glaucoma valve implant and with the time following implantation.

Chapter 12 presents the results of a prospective study on the development of ocular complications in children within 1 year after hematopoietic stem cell transplantation (HSCT) with emphasis on the risk of development of viral uveitis in immunocompromised children after HSCT.

Chapter 13 provides the English and Dutch summary with conclusions.

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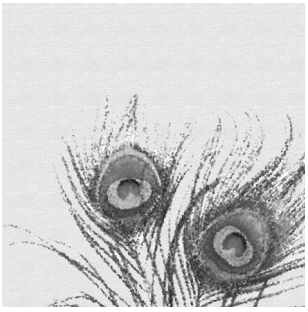

Chapter 2

Pathogenesis of uveitis associated with juvenile idiopathic arthritis

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Nico M. Wulffraat
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Submitted for publication

Abstract

Juvenile idiopathic arthritis (JIA) is the most common childhood rheumatic disease and the most prevalent systemic disorder in children with uveitis. The current prevailing opinion is that JIA is a multifactorial genetically predisposed autoimmune disorder that can be influenced by environmental factors and infections. However, the specific pathogenesis of JIA-associated uveitis is not understood, nor has the relationship between the eye and joint inflammation been established. Nevertheless, subtypes of JIA that are associated with uveitis, oligoarthritis, polyarticular rheumatoid factor negative and psoriatic arthritis appear to have common pathogenicity. This review summarizes our current knowledge regarding the pathogenesis of JIA-associated uveitis and discusses the possible role of immune responses and cytokine involvement, genetic associations and the influence of external triggers in this disease, an association that is supported by data obtained from experimental uveitis models.

Introduction

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease and the most prevalent systemic disorder in children with uveitis. The risk of developing uveitis for children with JIA is similar among children of European and non-European descent,[1;2] although the referral centers in Europe, North America and Israel report that the prevalence of JIA-associated uveitis can range from 15 to 67%.[3-7] JIA-associated uveitis typically presents as a chronic bilateral anterior uveitis with an initially silent course; however, during the course of the disease, a high risk of complications, including visual impairment, develops.[4;5;8-10]

JIA is not a single disease entity, but is actually a heterogeneous group of clinically distinct arthritic disorders that affect children under the age of 16 and persists for a minimum of six weeks.[11;12] According to the International League of Associations for Rheumatology (ILAR) criteria, JIA can be classified into seven subtypes (Table 1).[12] JIA subtypes that are associated with uveitis include oligoarthritis, polyarticular rheumatoid factor negative, and psoriatic subtypes (Table 1).[1;2;13] These subtypes share certain phenotypical features such as asymmetric arthritis, an early age of onset, female predisposition and anti-nuclear antibody (ANA) positivity. The common clinical features suggest that these entities have a common pathogenesis and may be distinct from the other JIA subtypes, a hypothesis that is supported at both the molecular and genetic levels.[13-17]

The current prevailing opinion is that JIA is a multifactorial autoimmune disorder to which patients are genetically predisposed and may be influenced by environmental factors and infections.[13;17-19] However, both the pathogenesis of JIA-associated uveitis and the relationship between the eye and joint inflammation are currently poorly understood. Uveitis can precede the onset of arthritis by several years, and conversely, arthritis can precede the onset of uveitis.[9] Why only approximately 30% of JIA patients develop uveitis is also currently unknown. The purpose of this review is to summarize the current data regarding the pathogenesis of JIA-associated uveitis and discuss the putative roles of immune responses and cytokines, genetic associations and the influence of external triggers, which are supported by data obtained using experimental models of uveitis.

Table 1. Classification of Juvenile Idiopathic Arthritis (JIA) according to International League of Associations for Rheumatology (ILAR) criteria.

Type of JIA [Ref 12]	**Definition** [Ref. 12]	**Incidence of chronic silent anterior uveitis** [Ref 1]
1. Systemic arthritis	≥ 1 joint, with fever and other systemic symptoms (evanescent erythematous rash; generalized lymph node enlargement; hepatomegaly and/or splenomegaly; serositis)	Rare
2. Oligoarthritis a) Persistent b) Extended	1- 4 joints during the first 6 months of the disease ≤4 joints throughout the disease course >4 joints after the first 6 months of disease	13-45%
3. Polyarthritis (rheumatoid factor-negative)	≥5 joints during the first 6 months of the disease	10%
4. Polyarthritis (rheumatoid factor-positive)	≥5 joints during the first 6 months of the disease	Very rare
5. Psoriatic arthritis	Arthritis and psoriasis, or arthritis and at least 2 of the following: - Dactylitis - Nail pitting or onycholysis - Psoriasis in a first-degree relative	10-20%
6. Enthesitis-related arthritis	Arthritis and/or enthesitis with at least 2 of the following: - The presence of (or a history of) sacroiliac joint tenderness and/or inflammatory lumbosacral pain - The presence of HLA-B27 antigen - Onset of arthritis in a male over 6 years of age - Acute (symptomatic) anterior uveitis - History of ankylosing spondylitis, enthesitis-related arthritis (ERA), - sacroiliitis with inflammatory bowel disease, Reiter syndrome, or acute anterior uveitis in a first-degree relative	Acute symptomatic) anterior uveitis
7. Other arthritis	Arthritis that does not meet any criteria or meets the criteria of more than one of the above categories	Rare, no precise data

Pathogenesis

1. Autoimmune hypothesis

Uveitis has been traditionally considered to be an autoimmune disorder caused by a loss of immune tolerance to autoantigens and the subsequent activation of autoreactive T lymphocytes.[20-24] Insight into intraocular immunological processes has been based primarily on studies using animal models, which have yielded valuable information regarding the pathogenesis of uveitis.

In general, the immune system's response to a stimulus can be divided in adaptive (antigen-specific) and innate (non-specific) immune responses. The prevailing hypothesis posits that both adaptive and innate immune responses are involved in the induction of autoimmune disorders, including uveitis. Indeed, innate and adaptive responses are intertwined, as activation of the adaptive immune response by T-lymphocytes may require prior stimulation of the innate immune response.[21;24;25] Moreover, in the pathogenesis of JIA, both adaptive and innate immunity may be involved in the inflammatory process, as mixed inflammatory infiltrates (e.g., innate and adaptive immune cells) have been identified in affected joints, and systemic neutrophil activation has been observed in distinct stages of the disease.[13;17;26-31]

1.1 T cells: the key orchestrators

T cells are considered to be the key cellular players in both non-infectious uveitis and JIA. Specifically, CD4+ T cells play a central role in the generation of the adaptive immune response, serving as both effectors and regulators of the inflammatory process.

1.1.1 Autoimmune uveitis

The currently identified subsets of CD4+ cells (T helper 1 (Th1), Th2, Th17 and T regulatory cells) and their putative roles in autoimmune uveitis are presented in Table 2.[25;32-34] The key role of pathogenic Th1 and Th17 phenotypes, which orchestrate intraocular inflammation in non-infectious uveitis, has been confirmed by studies of both animals and human patients.[20-22;35-38] On the other hand, T regulatory cells (Tregs; CD4+/CD25+/FoxP3+) seem to play a protective role, as they can attenuate a variety of physiological and pathological immune responses. Furthermore, Tregs play an important regulating role in the activation of Th1 and Th17 cells in uveitis as shown by studies of both mice and humans.[39-42] In contrast, the role of Th2 cells in non-infectious uveitis is less clear (Table 2).[32] CD8+ T cells do not appear to be necessary for the development of uveitis in experimental models.[20]

Table 2. CD4+ T-cell subsets and their putative role in autoimmune uveitis.

T cells	Subset	General functions [Ref 25;33;34]	Main produced cytokines [Ref 25;32-34]	Possible role in autoimmune uveitis [Ref. 32]
CD4+ (MHC class II restricted)	T helper 1 [Ref 25; 32;33]	- Cell-mediated immunity (bind to antigen presented by antigen-presenting cells (APCs) like phagocytic macrophages and dendritic cells. Results in inflammation. - Clearance of intracellular pathogens - Inhibition of Th2 and Th17 Pathologic response: organ specific autoimmunity	IFN-γ* IL-2 TNF-α	Pathogenic Protective?*
	T helper 2 [Ref 25; 32;33]	- Extracellular immunity (promotes B-cell proliferation and immunoglobulin production. Class switching to IgE.) - Clearance of helmints - Inhibition with Th1 and Th17 Pathologic response: asthma and allergy	IL-4 IL-5 IL-10 IL-13	Pathogenic Protective
	T helper 17 [Ref 32;33]	- Cell-mediated immunity - Clearance of extracellular pathogens Pathologic response: organ specific autoimmunity	IL-17 IL-21 IL-22 TNF-α	Pathogenic
	T regulatory [Ref 32;34]	- Maintenance of self-tolerance and immune homeostasis - Suppress wide range of immune cells (including CD4+ and CD8+ T cells, B cells, APCs and others) Dysfunction causes autoimmune diseases, immunopathology and allergy.	IL-10 TGF- β	Regulatory

* Production of IFN-γ early in the response (primarily from innate immune cells) reveals protective properties in experimental autoimmune uveitis.[Ref. 32]

1.1.2 JIA-associated uveitis

The precise role of intraocular T cells has not been studied systematically in children with JIA-associated uveitis. To date, only one published study has reported an immunohistochemical analysis of an enucleated globe obtained from a patient with end-stage JIA–associated uveitis, in which only rare CD4+ and relatively few CD8+ cells were observed; in contrast, plasma cells and B cells were the most abundant cell types in the inflammatory infiltrate.[43] The enucleated globe was obtained from a patient who had been treated using intensive immunosuppressive medication and anti-TNF-alpha agents, which might have influenced cellular infiltration into the eye. Also the resulting histopathological picture from an enucleated globe might not accurately represent the earlier stages of inflammation; therefore, additional immunohistochemical studies of JIA-associated uveitis specimens obtained at various stages of the disease are needed to test this hypothesis further.

1.1.3 Arthritis

Similar concepts regarding the role of T cells are also being investigated with respect to oligo-/polyarticular JIA, in which an imbalance between pro-inflammatory Th1/Th17 cells and anti-inflammatory Tregs is believed to underlie the joint inflammation.[13;15;17;44] With respect to arthritis, histological analyses have revealed a high proportion of T cells, with a significant predominance of CD4+ cells, in the synovium.[17;26;29-31;45] High numbers of Th17 cells were found in the joints of children with JIA; these numbers were higher in children with extended oligoarthritis than in children with persistent oligoarthritis.[46] Although the precise mechanism(s) that Tregs use in human patients still remain to be delineated, their regulatory role in JIA has been demonstrated.[47;48]

1.2 Soluble mediators of inflammation: cytokines, chemokines and other mediators

Soluble mediators of inflammation—including cytokines—regulate immune cells during the inflammatory process. Cytokines are produced by a wide variety of cell types within the immune system and form a complex dynamic pattern that drives the immune response in either the inflammatory or anti-inflammatory direction.

1.2.1 Uveitis

Studies of soluble mediators of inflammation in the aqueous humor (AH) of patients with JIA-associated uveitis have revealed the involvement of a broad spectrum of cytokines with no clear pattern or predominance of a discrete subset of T cells.[49]
Patients with JIA-associated uveitis significantly higher levels of IL-2, IL-6, IL-13, IL-

18, IFN-γ, TNF-α, sICAM-1, RANTES and IP-10 than non-inflammatory pediatric controls.[49] This finding reflects an increase in cytokines that are produced by both Th1 (e.g., IFN-γ) and Th2 (e.g., IL-13) cells, as well as activated macrophages and other cells in the immune system. However, these increased cytokine levels are not specific to JIA-associated uveitis, as they have also been found in patients with other pediatric uveitis entities. No difference was found in the expression of IL-10 (which is produced by Tregs) between patients with JIA-associated uveitis and control subjects. Moreover, an intraocular T cell phenotype specific to JIA-associated uveitis was not identified in these samples; however, the samples were collected at various stages of the disease.

1.2.2 Arthritis

A wide variety of soluble modulators such as IL-6, TNF-α, IL-10 and macrophage migration inhibitory factor (MIF) have been implicated in the pathogenesis of JIA.[44] For example significant increases in both Th1-related (IL-6, IL-15, CCL2, CCL3, CXCL8, CXCL9 and CXCL10) and Th2-related (CCL11 and CCL22) markers have been found in the synovial fluid of patients with JIA.[14] In addition, the Th17 subtype effector cytokine IL-17 was increased in the plasma of children with oligoarticular or polyarticular JIA.[14]

1.3 B cells: participants with a less clear role

1.3.1 Uveitis

Unlike the role of T cells, the specific role of B cells in JIA-associated uveitis is much less clearly understood. Uveitis is generally considered to be a T-cell-mediated disorder, and it is striking to note that both histological case reports of enucleated eyes and an iridectomy specimen revealed an initial predominance of plasma cells (terminally differentiated B cells) followed by a predominance of CD20+ cells (B cells) infiltrating the eye.[43;50-52] Together, these studies suggest the active production of immunoglobulins (Ig) within the eye; indeed, IgG, IgM and IgA were found, albeit inconsistently, in the inflammatory infiltrate.[43;50;51] However, these findings were based primarily on enucleated eyes, thus representing the end-stage of the disease process. Whether B cells and plasma cells are also present in similar amounts in earlier stages of the disease is not currently known.

JIA-associated uveitis is commonly identified by ANA positivity, which reflects the production of abnormal self-targeting antibodies in this disorder; however, the nature of the self-antigen remains unknown, and the role of these antibodies in the pathogenesis of the disease is still unclear.

1.3.2 Arthritis

Together with T cells, B cell aggregates and plasma cells have been detected in the synovium of patients with arthritis.[26;30;45;53] The infiltration of plasma cells into the synovium correlates with ANA positivity.[45] An expansion of IgG-secreting B cells in synovial fluid and increased expression of CD86 (which is expressed on antigen-presenting cells) suggest a dual role for B cells in JIA, on one hand, they act as antibody-producing cells, and on the other hand, they act as antigen-presenting cells.[53] In addition, peripheral blood mononuclear cells from patients with early-onset disease have higher expression of B cell specific genes than genes that are specific to cells in the myeloid lineage.[54] In addition, hypergammaglobulinemia consistent with B cell hyperactivity has also been reported in JIA patients.[55]

In summary, these results suggest that the role of B cells in the pathogenesis of JIA-associated uveitis may be more central than was originally believed, and the mechanism(s) through which B cells act may be based on both the production of autoantibodies and the presentation of antigens to T cells. This hypothesis provides a rationale for an experimental therapy using the anti-CD20 monoclonal antibody Rituximab, which was recently proposed as a possible "rescue therapy" option for treating both refractory uveitis and arthritis in JIA patients, although its use in treating oligoarticular and poliarticular arthritis has been very limited.[56-58]

1.4 Granulomatous versus non-granulomatous uveitis

JIA-associated uveitis is generally considered to be a non-granulomatous entity.[1;2] Although most histological studies have confirmed this notion,[43;50;51] one study showed the presence of epithelioid cells and giant cells in a patient with clinically non-granulomatous JIA-associated uveitis.[52] However, as demonstrated recently, without an immunohistochemical analysis of these specimens, ciliary epithelial cells can be misidentified as epithelioid and giant cells.[43] Moreover, another study reported that the prevalence of clinically granulomatous uveitis among children with JIA is higher (28%) than reported previously, particularly among African-Americans.[59]

2. Genetic basis of JIA-associated uveitis

JIA is a complex genetic trait, meaning that its onset can be influenced by a multitude of genetic and environmental factors.[17] Among monozygotic twins, the concordance rate for JIA-associated uveitis is 25%, and the concordance rate for arthritis is 20-40%; in contrast, parent-offspring pairs with JIA and extended multiplex JIA families are relatively uncommon.[17;60-66] A large Finnish sibling cohort exhibited a higher rate of

uveitis among siblings than in the general JIA population (26 vs. 16%, respectively), although this difference did not reach statistical significance.[65]

2.1 HLA genes

Although many genetic associations have been reported for JIA, the specific susceptibility to JIA-associated uveitis has been studied less extensively. Most JIA-associated genes are located in the human leukocyte antigen (HLA) region, which is divided into two classes of genes, class I genes (HLA A, B, and C) and class II genes (HLA-DR, DP, and DQ). The HLA region is located on chromosome 6 and encodes cell-surface antigen-presenting proteins as well as many other genes that are important for immune system functions. Class I molecules are expressed by nearly all nucleated cells, whereas class II molecules are expressed predominantly by antigen-presenting cells (dendritic cells, macrophages, and B-lymphocytes).

Genetic associations with JIA-associated uveitis have been identified for both HLA class I and II genes; however, the outcomes of these associations are inconsistent. Studies in Europe report an increased risk of uveitis associated with HLA-A19, HLA-B22[67] and HLA-DRB1*13 (which is a split antigen of the older HLA-DR6 serotype group)[68]; however, the latter association was not supported by a previous European study.[69] An association between uveitis and HLA-DRB1*11 (a split antigen of HLA-DR5) has been demonstrated by both American[70-72] and European[73] studies, albeit not consistently.[69] Interestingly, a protective role for HLA-DR1 has been confirmed by both European and American studies.[67;69-72] Numerous HLA class II associations between JIA-associated uveitis and oligoarticular and polyarticular JIA in general (HLA-DRB1 and HLA-DP) underscore the importance of CD4+ T cells, which are HLA-class II restricted, in their pathogenesis (Table 2).[17-19;69;74;75] Thus, to date, only the protective role of HLA-DR1 has been confirmed independently by multiple studies.

2.2 Non-HLA genes

Non-HLA genes also appear to play a role in JIA; however, their precise role has not yet been elucidated. Although associations between JIA and *PTPN22*, *TNFA*, *MIF*, *WISP3* and *SLCIIA1* have been confirmed independently, the relationship between these genes and uveitis is currently unknown.[17;18;76]

3. JIA-associated uveitis and external triggers

Multiple external (i.e., non-genetic) factors have been suggested as possible triggers of JIA. If JIA were solely a genetically determined disease, even a complex disease driven

by multiple interacting genes, the concordance rate among monozygotic twins would approach 100%. However, as discussed above, this is clearly not the case. In the search for external JIA triggers, infections, vaccines and environmental factors have all been studied; however, to date, no definitive causal relationship has been established. Table 3 summarizes our current knowledge of external triggers in JIA.

3.1 Infections

The current theories regarding the putative association between infections and JIA are based on the hygiene hypothesis and molecular mimicry mechanisms. The hygiene hypothesis states that a lack of exposure to infections in early childhood can increase the risk of developing an autoimmune disease due to insufficient regulatory T cell formation, which can lead to an over-activation of the Th1 pathway.[77] The molecular mimicry theory states that specific infectious antigens can exhibit similarities with self-antigens, causing an autoimmune reaction even years after the original infection.[78] Currently, it is not clear which, if any, of these principles influences the inflammatory process in JIA.

3.1.1 Bacterial infections

Infections in the first year of life that require hospitalization have been suggested to increase the risk of JIA later in life.[79] A serological study investigated a wide range of putative causative bacterial infections and detected seropositivity in nearly 43% of the patients with JIA compared to only 8% of the control group; *Mycoplasma pneumoniae* was the most common infectious agent in these patients.[80] Streptococcal and *Borrelia burgdorferi* (Lyme disease) infection can cause both inflammation of the large joints and uveitis; however, these conditions do not seem to progress to JIA, and the clinical picture of uveitis is distinct from uveitis associated with JIA.[81-87] On the other hand, some evidence suggests that a streptococcal infection can cause flares in the disease course of arthritis in JIA patients.[88]

3.1.2 Viral infections

3.1.2.1 Parvovirus

Parvovirus B19 (B19V) causes childhood erythema infectiosum (also known as the fifth disease), and a role for B19V in the pathogenesis of JIA has been suggested. Approximately 8% of children with fifth disease develop joint symptoms, and 27% of children who develop joint disease following a B19V infection eventually meet the criteria for JIA.[89] Because B19V infection does not always give rise to typical fifth disease symptoms, the true prevalence of JIA following an asymptomatic infection could be

Table 3. External triggers and their relationship to Juvenile Idiopathic Arthritis (JIA) and to uveitis in general.

	Infectious agent/ Factor	Association		Reference(s)
		JIA[a]	Uveitis in general	
Infections	Parvovirus B19	++	+	*89-94*
	Influenza virus	+	+[b]	*102-104*
	M. pneumoniae	+	?	*80*
	Streptococcus	+/-	?	*83; 88*
	Epstein-Barr virus	+/-	++	*95; 96; 98; 99*
	Rubella virus	+/-	++	*96; 105-11*
	B. burgdorferi	-	++	*81; 82; 84; 86; 87*
Environmental factors	Hospitalization within the 1st year of life	+	?	*79*
	Psychological stress	+	+	*132; 133*
	Vitamin D	+	+	*127-131*
	Prolonged pregnancy	+	?	*79*
	Birth by Cesarean section	+	?	*79*
	High Apgar score	+	?	*79*
	Air pollution	+	?	*125*
	Maternal smoking	+/-	?	*79; 120*
	Breastfeeding	+/-	?	*122-124*
	Vaccinations	-	++	*100; 101; 107; 112-118*
	Animal exposure	-	?	*126*
	Birth order	-	?	*79; 119*

[a]: no associations have been independently confirmed in large studies

[b]: case reports

JIA: Juvenile Idiopathic Arthritis; ++: association suggested in several studies; +: association suggested in a single study or case reports; +/-: controversial results in distinct studies; ?: no data available

even higher. The presence of both anti-B19V IgM and B19V DNA in the sera of patients with JIA suggests that B19V may be involved in the pathogenesis of JIA. However, the available data regarding anti-B19V IgG in JIA are inconsistent.[90-92] Elevated levels of IgG against the B19V non-structural protein 1 (NS1) have been measured in children with rheumatic disease.[93] Interestingly, an increased prevalence of anti-NS1 IgG has also been found in the sera of patients with endogenous uveitis,[94] and ten percent of the patients in this study had JIA-associated uveitis. In addition, we have detected the production of intraocular IgG against Parvovirus B19 in a significant percentage of B19V-seropositive children with JIA and uveitis (de Groot-Mijnes et al., manuscript in preparation), which further supports the hypothesis that this virus may play a role in both JIA and JIA-associated uveitis.

3.1.2.2 Epstein-Barr virus

The role of EBV in JIA-associated uveitis is currently a subject of controversy. Interestingly, three HLA peptides with sequence homology to EBV peptides have been associated with oligoarticular JIA, suggesting that EBV might serve as a factor in the pathogenesis of JIA.[95] However, among patients with general uveitis (i.e., not associated with JIA), the presence of intraocular EBV DNA is a relatively frequent and non entity-specific finding.[96-99]

3.1.2.3 Influenza virus

Despite occasional reports that uveitis may be related to Influenza viruses and Influenza virus vaccination, it remains unknown whether there is any connection between these viruses and the development of uveitis in children with JIA.[100-103] However, children with JIA who were born during an epidemic of Influenza virus A/H2N2 developed arthritis during a subsequent flu epidemic following exposure to a related influenza A subtype H3N2. It has been suggested that these children had been presensitized to Influenza A their prior *in utero* contact with the first strain.[104] Serological analyses revealed that these children carried a higher antibody titer to Influenza virus A/H2N2 than children who were born during the same epidemic but did not develop arthritis.

3.1.2.4 Other viral infections

There is no clear evidence supporting the involvement of other viral infections in JIA uveitis (Table 3). Although the prevalence of Rubella virus might be increased among patients with JIA, the results are inconclusive.[105;106] Recently, a large Dutch study reported lower concentrations of vaccine-specific antibodies against mumps, Rubella, diphtheria and tetanus in JIA patients relative to controls.[107] These decreased titer lev-

els may reflect a weaker immune response to vaccination in JIA patients, possibly resulting in higher infection rates compared to healthy controls. However, Rubella virus is associated with Fuchs heterochromic uveitis, which has a clinical presentation that is distinct from JIA-associated uveitis.[96;108-111]

On the other hand, antibodies against measles are elevated in JIA patients.[107] Several published case reports have described the development of uveitis following a vaccination against Influenza virus, Hepatitis B virus, Varicella zoster virus, or a combined vaccine against measles, mumps and rubella (MMR); however, a causal relationship has yet to be established.[100;101;112-116] Vaccinations are unlikely to serve as a risk factor for exacerbating JIA, as both the MMR and the meningococcal C vaccine have no influence on either JIA disease activity or JIA medication use.[117;118]

3.2 Environmental factors

3.2.1 Perinatal factors and breastfeeding

Borderline associations have been found between JIA and prolonged pregnancy (i.e., birth at gestational age >42 weeks) or delivery by Caesarean section, and a marginally significant decreased risk of developing JIA with an Apgar score of ≤6 at postnatal five minutes.[79] In contrast, no association has been identified with respect to the number of older siblings, birth order, birth weight, civil status, multiple births, malformations, season of birth, maternal-child blood group incompatibility or maternal infection.[79;119] Whether prenatal smoking plays a role is controversial.[79;120] Although smoking cigarettes appears to be a risk factor for non-infectious uveitis,[121] it is unclear whether any of the aforementioned factors can also influence the development of JIA-associated uveitis. Currently, there is no information regarding a relationship between JIA-associated uveitis and breastfeeding. Although studies of a putative relationship between breastfeeding and arthritis have yielded inconsistent results, children with oligoarticular disease appear to have breastfed for a briefer period of time.[122-124]

3.2.2 Air pollution and animal exposure

A recent study to examine the relationship between air pollution and the development of JIA found that preschool-age children which higher concentrations of PM-2.5 (particulate matter that is smaller than 2.5 micrometers in diameter and can pass through the lungs) in the two weeks prior to JIA onset had an increased risk of developing the disease.[125]

Contact between young children and animals has been found to confer protection against autoimmune diseases such as inflammatory bowel disease and systemic lupus

erythematosus, although no relationship has been found between living on a farm and developing oligoarticular JIA.[126] The relationship between these factors and uveitis remains unknown.

3.2.3 Vitamin D and sun exposure

Some evidence suggests that active vitamin D (1,25-OH_2D_3 or calcitriol) can inhibit Th17 cell production and can protect against autoimmunity.[127] This finding suggests that exposure to the sun can help to protect against the development of JIA-associated uveitis. Moreover, calcitriol has been found to suppress autoimmunity by inhibiting the Th17 response in a mouse model of experimental autoimmune uveitis.[128] The relationship between vitamin D and JIA was investigated in a study that found that 55% of patients have low levels of 25-hydroxyvitamin-D (25(OH)D); however, there was no direct correlation with disease activity.[129] In addition, the season of birth, which is related indirectly to sun exposure, was not found to be associated with the progress of JIA.[79] The relationship between exposure to the sun or vitamin D and JIA-associated uveitis is not known; however, a positive correlation between vitamin D deficiency and the development of some other types of uveitis (e.g., Vogt-Koyanagi-Harada uveitis and uveitis in ankylosing spondylitis) has been identified from clinical studies.[130;131]

3.2.4 Stress and psychosocial factors

The influence of stress on the development of JIA-associated uveitis is currently unknown. However, compared to controls, patients with non-JIA-associated acute anterior uveitis have more stress in the period preceding attacks. Moreover, stress levels were significantly lower in patients with resolved uveitis compared to the levels in patients with recurrent uveitis.[132] Although it was unknown whether the stress caused the uveitis, or vice versa, stressful life events were found to be a exacerbating factor in the development of JIA.[133] Compared to healthy controls, a significantly higher proportion of children with JIA have unmarried parents or were adopted.[133]

4. The eye and the joint: a link to be explored

The co-occurrence of inflammation in two different organs, the eyes and joints, suggests the activation of common pathogenic mediators and/or a common reaction to shared self-antigens. However, the biological basis underlying this phenomenon has not been explored fully. Some studies have found increased levels of antibodies against the low molecular-weight iris antigen (LMW-IA) and/or retinal S antigen in children with JIA and uveitis; however, these results were inconclusive.[134;135]

Conclusions

In summary, the complex pathogenesis of JIA-associated uveitis is poorly understood, although extensive studies of the mechanisms underlying arthritis and experimental uveitis have significantly increased our understanding of the processes behind this disease. All of the combined data point to the development of aberrant immune responses involving both the innate and adaptive immune responses. CD4+ T cells are the primary cell type underlying the pathogenesis of JIA-associated uveitis. In contrast, the role of B cells has been studied less extensively and is understood less, but these cells may also play a role in the pathogenesis of JIA. JIA-associated uveitis appears to have a genetic predisposition, particularly with respect to HLA class II genes; to date, however, only a protective role of HLA-DR1 has been confirmed independently by multiple studies. JIA-associated uveitis has a relatively low concordance rate among twins, which suggests the involvement of external (non-genetic) factors. Although many external factors, including a variety of infections, have been proposed to increase the risk of developing JIA, none of these factors has been independently and adequately confirmed. The development of chronic and recurrent inflammation in the eyes and joints suggests a similarity between these two organs, although this hypothesis has not yet been tested.

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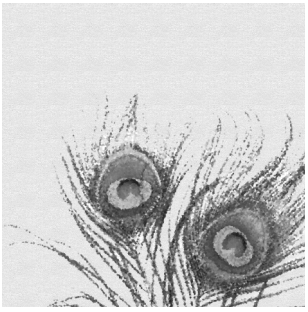

Chapter 3

Male gender as a risk factor for complications in uveitis associated with juvenile idiopathic arthritis

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Abstract

Purpose: To analyze the role of baseline factors in long-term development of ocular complications in uveitis associated with juvenile idiopathic arthritis (JIA).
Design: Retrospective nonrandomized interventional case series.
Methods: Data of 117 affected eyes (65 patients) with JIA-associated uveitis with a minimum follow-up of 1 year were obtained. Development of complications was analyzed univariately and multivariately in relation to gender, age of onset of uveitis (<7 years or >7 years), and initial manifestation of JIA (as uveitis or as arthritis).
Results: Female-to-male ratio was 3:1 and follow-up for uveitis ranged from 1.1 to 27.5 years (median 7.6 years). Time interval between arthritis and uveitis was shorter in boys (median 0.3 year) than in girls (median 1.0 year) ($P < 0.01$). At 5 years of follow-up boys suffered more frequently from cystoid macular edema (CME) (50% vs 4%; $P < 0.01$) and papillitis (31% vs 2%; $P < 0.01$), and needed more cataract surgery (59% vs 32%; $P = 0.02$). At 5 years of follow-up children with initial uveitis had more posterior synechiae, band keratopathy, and CME (all $P < 0.02$), but less glaucoma ($P = 0.03$). In multivariate analysis male gender appeared to be independently associated with cataract surgery (adjusted hazard ratio [HR] = 4.33; $P < 0.01$), CME (HR = 4.59; $P = .01$), and papillitis (HR = 4.10; $P = 0.01$). Development of posterior synechiae was independently associated with initial uveitis (HR = 3.21; $P < 0.01$).
Conclusions: Male gender and uveitis as initial manifestation of JIA were independently associated with a complicated course of JIA-associated uveitis. Age of onset of JIA-associated uveitis does not seem to have independent prognostic value for the course of this ocular disorder.

Introduction

Silent, chronic anterior uveitis is a common complication of juvenile idiopathic arthritis (JIA). The uveitis may follow a severe course and complications are encountered frequently. Uveitis complicates the course of JIA in up to 38% of patients and it is the major systemic disease association of uveitis in childhood.[1;2]

The current screening program is based on known risk factors for the development of uveitis in JIA (female gender, oligoarticular type of arthritis, seropositivity for antinuclear antibody [ANA], and onset of arthritis before the age of 6 years).[1;3–5] However, despite intensive screening, uveitis in JIA can be worsened over time by many potentially sight-threatening complications such as cataract, secondary glaucoma, cystoid macular edema (CME), papillitis, band keratopathy, and posterior synechiae.[1;2;4;6–9] Vision-disturbing complications at young age can also lead to amblyopia, a unique complication in young children.

Prognostic factors in JIA-associated uveitis are not clearly identified and the results of the studies are often controversial. Different studies pointed out that several factors could be associated with poor prognosis, including short intervals between the diagnosis of arthritis and uveitis,[9–12] severity of uveitis at first examination,[6;13] and male gender.[6;11–13] However, gender as a prognosticator of severity or outcome of uveitis could not be confirmed by other authors.[7;9;14] Young age at onset of ocular disease has been suggested as a negative prognostic factor for JIA-associated uveitis,[6;9;12] but other authors could not confirm this correlation.[7;13]

The purpose of this study was to evaluate the role of gender, age of onset of uveitis, and initial manifestation of JIA (as initial arthritis with later uveitis or initial uveitis with later arthritis) as prognostic factors of JIA-associated uveitis.

Patients and Methods

The medical records of 65 pediatric patients (117 affected eyes) with JIA-associated uveitis were reviewed. All of these children were examined between 1981 and 2008 at the Department of Ophthalmology, University Medical Centre, Utrecht, the Netherlands. All children with onset of JIA-associated uveitis before 16 years of age and a minimum follow-up of 1 year were included in the study. The diagnosis of JIA was based on the criteria of the International League against Rheumatism.[15;16] All patients were seen by a pediatric rheumatologist who confirmed the diagnosis of JIA. Patients

with JIA were being referred to our clinic for ophthalmologic screening according to the guideline of American Academy of Pediatrics.[17]

As a tertiary uveitis center, we also get referrals of children with uveitis from primary and secondary ophthalmologic care. Some of these children were diagnosed primarily with uveitis and only secondly with JIA, sometimes years after the diagnosis of uveitis. Evaluation of uveitis was made according to the criteria of the International Uveitis Study Group.[18] Uveitis was diagnosed if 1+ or more cells in the anterior chamber were seen over at least 2 eye examinations. We considered uveitis as chronic if the duration of the active ocular inflammation was longer than 3 months.

The following information from the medical data of the patients was recorded: date of birth, gender, date of onset of uveitis, date of diagnosis of JIA, location and course of uveitis (acute or chronic), laterality, ocular complications, surgical procedures, treatment, and follow-up. The time interval between arthritis and uveitis was calculated from the date of diagnosis of arthritis to the date of diagnosis of uveitis. Laboratory results of antinuclear antibodies and HLA-B27 were also registered. The following ocular complications were registered: cataract requiring surgery, secondary glaucoma, posterior synechiae, band keratopathy, CME, papillitis, strabismus, and amblyopia. Because of difficulties in objective measurement of cataract formation we used cataract surgery as a measure for cataract. Secondary glaucoma was defined as the presence of pathologic cupping of the optic disc seen by ophthalmoscopy and/or a glaucomatous visual field defect, in combination with intraocular pressure (IOP) higher than 21 mm Hg, or both.[19] Ocular hypertension without the presence of pathologic disc cupping and/or glaucomatous visual field defect was not scored. CME was defined as the presence of macular thickening with cyst formation observed by indirect ophthalmoscopy or by optical coherence tomography (OCT).

Slit-lamp and fundus examination were performed by an ophthalmologist specialized in pedriatic uveitis. Additionally, all children in our clinics were being seen by an orthoptist at the initial visit and regularly during the follow-up to detect development of strabismus and amblyopia. Patients were subdivided according age at onset of uveitis into a younger onset (uveitis onset <7 years of age) or older onset group (uveitis onset >7 years of age). Two different types of initial manifestation of JIA were distinguished: initial manifestation as arthritis with later diagnosis of uveitis (classic); and initial manifestation as uveitis followed later by development of arthritis, making the diagnosis of JIA possible. Children in whom the signs of uveitis were present at initial eye screening after the diagnosis of JIA were considered as patients with classic manifestation of JIA.

The use of topical (anti-inflammatory, long-term mydriatics), periocular (perioperative, therapeutic), and systemic therapy (corticosteroids and immunomodulatory therapy) were noted.

Statistical analysis of the data was performed with SPSS 15.0.1 (SPSS Inc, Chicago, Illinois, USA). We performed the analysis "by eye," including all affected eyes in the analysis. However, analysis "by patient" was used in comparison of baseline constant host characteristics between subgroups: laterality, ANA status, administration of systemic therapy, and duration of follow-up. Statistical analysis of ocular complications was performed "by eye" with correction for paired eyes in the analysis.

The Pearson X^2 test or Fisher exact test was used for univariate analysis of categorical variables. Mann-Whitney U test was used to compare means of not normally distributed variables. Cox proportional hazard regression with correction of standard error with clustering using robust method was applied in multivariate analysis to identify independent predictive factors of unfavourable prognosis.[20] Baseline variables with $P \leq 0.05$ in univariate analysis and clinically significant potential confounders were entered into the multivariate analysis. For the multivariate analysis of ocular complications we entered age of onset of uveitis (as a numeric variable), gender, initial manifestation of JIA, ANA serologic status, presence of posterior synechiae at first visit to an ophthalmologist (except for analysis of risk factors for development of posterior synechiae themselves), and time between the diagnosis of arthritis and uveitis into the Cox proportional hazard regression model. All multivariate models were adjusted for therapy administration (periocular injections, systemic corticosteroids, and immunomodulatory therapy). P values of less than 0.05 were considered statistically significant. All significances were 2-tailed. In the presentation of the results we used the mean if the data were normally distributed (by Kolmogorov-Smirnov test; $P = 0.05$) and median if not normally distributed.

Results

General characteristics of the study population

Of 65 patients, 117 eyes were affected by uveitis. Bilateral disease was observed in 52 of 65 patients (80%). We observed the typical manifestation as silent anterior uveitis in 116 eyes (99%) and panuveitis in 1 eye (1%). One HLA-B27-positive patient with unilateral disorder had acute uveitis, while all other patients suffered from chronic anterior uveitis. Time between the diagnosis of arthritis and uveitis varied, with a

TABLE 1. General Characteristics of Patients With Uveitis Associated With Juvenile Idiopathic Arthritis According to Age of Uveitis Onset, Gender, and Initial Manifestation of Juvenile Idiopathic Arthritis

	Age of Uveitis Onset			Gender			Initial Manifestation of JIA		
Characteristics	0–7 Years N = 48 (%)	7–16 Years N = 17 (%)	*P* Value	M N = 18 (%)	F N = 47 (%)	*P* Value	Uveitis N = 15 (%)	Arthritis N = 50 (%)	*P* Value
Females, n (%)	35 (73)	12 (71)	>.99	NA	47 (100)	NC	7 (47)	40 (80)	**.02**[b]
Age of onset, median (range)	NC	NC	NC	4.3 (1.7–13.8)	4.2 (1.7–16.0)	.48	4.0 (1.7–12.1)	4.5 (1.5–16.0)	.68
Bilateral, n (%)	42 (88)	10 (59)	**.03**[b]	16 (89)	36 (77)	.33	14 (93)	38 (76)	.27
Time between diagnosis of arthritis and uveitis,[a] median (range)	0.7 (0–6.7)	2.0 (0–12.3)	**.02**[b]	0.3 (0–5.0)	1.0 (0–12.3)	**.02**[b]	0.3 (0.1–7.3)	0.8 (0–12.3)	.86
Uveitis as initial manifestation of JIA	12 (25)	3 (18)	.74	8 (44)	7 (15)	**.02**[b]	15 (100)	NA	NC
Follow-up,[a] median (range)	8.3 (1.1–27.5)	7.2 (1.2–23.1)	.51	11.0 (1.1–27.5)	7.2 (1.1–22.9)	**.05**[b]	12.6 (1.1–27.5)	6.1 (1.1–23.1)	.07
ANA-seropositive	42 (91)	13 (81)	.36	16 (94)	39 (87)	.66	11 (73)	44 (94)	**.05**[b]
Systemic corticosteroids	18 (38)	8 (47)	.53	8 (44)	18 (39)	.78	10 (67)	16 (33)	**.02**[b]
Immunomodulatory therapy	39 (81)	12 (71)	.49	15 (83)	36 (77)	.74	12 (80)	39 (78)	>.99

F = female; JIA = juvenile idiopathic arthritis; M = male; NA = not applicable; NC = not comparable.
[a]Time is presented in years.
[b]Significant *P* values are highlighted in bold.

median of 0.7 years (range 0 to 12.3 years; Table 1). Fifty-five patients (85%) were ANA positive and in 3 patients ANA status was unknown. Follow-up of the whole group ranged from 1.1 to 27.5 years with median of 8.2 years.

Age of onset of uveitis. Age of onset of uveitis varied from 1.5 to 16 years (median 4.2; Table 1). Bilateral disease was more frequent in the group with the uveitis onset < 7 years of age: 42 of 48 patients (88%), versus 10 of 17 patients (59%) in the older-onset group (P = 0.03). The time interval between the diagnosis of arthritis and uveitis was shorter in the younger-onset group (P = 0.02) (Table 1). There were no significant differences between 2 age groups in gender distribution, duration of follow-up, ANA serologic status, and treatment strategies (Table 1).

Gender. The female-to-male ratio in the whole group was 3:1. General characteristics of the study population according to gender subgroups are provided in Table 1. Time between the diagnosis of arthritis and uveitis was shorter in boys (0.3 years) than in girls (1.0 years; P = 0.02; Table 1). Additionally, boys presented with uveitis as initial manifestation of JIA more frequently than girls (44% of boys vs 15% of girls; P = 0.02; Table 1). No difference in HLA-B27 status was observed between boys and girls with initial uveitis.

Initial manifestation of juvenile idiopathic arthritis. Children initially presenting with uveitis were less frequently ANA positive (P = 0.05) (Table 1). They also needed

more intensive treatment with more frequent use of systemic corticosteroids (P = 0.02; Table 1).

Complications

Cumulative incidences of ocular complications according to baseline characteristics are presented in Table 2. Posterior synechiae were the most frequent complication at onset of uveitis and they were present in 12/117 (10%) of eyes (Supplemental Table 1). Nobody from our series presented with secondary glaucoma, CME or papillitis. Furthermore, no differences in frequencies of complications at onset of uveitis were observed between groups (Supplemental Table 1).

Age of onset of uveitis. Frequencies of complications from onset up to 10 years of follow-up did not differ between the patients with onset <7 and >7 years of age.

Gender. Boys exhibited more complications at different points of follow-up, including posterior synechiae, cataract surgery, band keratopathy, CME, papillitis, and amblyopia (Table 2). Posterior synechiae were more common in boys at up to 3 years of follow-up (50% vs 25%; all P ≤ 0.02; Table 2). CME occurred significantly more frequently in boys at all standard points of follow-up (all P ≤ 0.05) (Table 2, Supplemental Table 1). Papillitis was significantly more frequently seen in boys at up to 5 years of follow-up (31% vs 2%; P < 0.01) (Table 2). Boys underwent cataract surgery more frequently. After 3 years of follow-up 56% of boys' eyes were operated on for cataract in contrast with 9% of girls' eyes (P < 0.01). At 5 years of follow-up this difference was still significant (59% in boys vs 32% in girls; P = 0.02; Table 2).
Additional analysis of complication frequencies in boys and girls was performed within a group with classic manifestation of JIA only (manifestation with initial arthritis). This analysis still revealed significant differences in frequencies of CME, papillitis, and cataract surgery between boys and girls in disadvantage of boys (Supplemental Table 2).

Initial manifestation of juvenile idiopathic arthritis. The initial uveitis group was more frequently characterized by the presence of posterior synechiae, band keratopathy, cataract surgery, and CME (Table 2). Children with initial uveitis had significantly more posterior synechiae at all standard follow-up points (all P ≤ .02) (Table 2). Band keratopathy was also more frequent in the initial uveitis group during the first 5 years of follow-up (P ≤ 0.03; Table 2). CME was more often diagnosed in the group with initial uveitis; this difference was noted from 3 up to 10 years of follow-up (67% of the

TABLE 2. Cumulative Incidences of Ocular Complications of Uveitis Associated With Juvenile Idiopathic Arthritis After 1, 3, and 5 Years of Follow-up With 95% Confidence Intervals, According to Gender, and Initial Manifestation of Juvenile Idiopathic Arthritis[a]

Complications	Total Percentage of Affected Eyes (± 95% CI)	Gender			Initial Manifestation of JIA		
		M	F	*P* Value	Uveitis	Arthritis	*P* Value
First 1 year of follow-up							
No of patients = 65							
No of eyes = 117							
Secondary glaucoma (%)[b]	2 (± 2)	NA	2	>.99	NA	2	>.99
Cataract surgery (%)	6 (± 4)	6	6	>.99	3	7	.68
Posterior synechiae (%)	27 (± 8)	47	20	**<.01**[c]	62	16	**<.01**[c]
Band keratopathy (%)	5 (± 4)	12	2	.06	14	2	**.03**[c]
CME (%)	8 (± 5)	21	3	**<.01**[c]	11	7	.67
Papillitis (%)	4 (± 4)	12	1	.03	7	4	.60
Strabismus (%)	3 (± 3)	7	3	.32	9	3	.23
Amblyopia (%)	10 (± 5)	16	9	.31	19	8	.16
First 3 years of follow-up							
No of patients = 55							
No of eyes = 99							
Secondary glaucoma (%)	14 (± 7)	17	14	.76	4	18	.11
Glaucoma surgery (%)	8 (± 5)	10	8	.70	8	9	>.99
Cataract surgery (%)	24 (± 8)	56	9	**<.01**[c]	56	13	**<.01**[c]
Posterior synechiae (%)	31 (± 9)	50	25	**.02**[c]	64	22	**<.01**[c]
Band keratopathy (%)	14 (± 7)	27	9	**.03**[c]	40	6	**<.01**[c]
CME (%)	14 (± 7)	41	3	**<.01**[c]	29	10	**.04**[c]
Papillitis (%)	8 (± 5)	21	3	**.01**[c]	17	6	.20
Strabismus (%)	4 (± 4)	12	2	.10	14	2	**.05**[c]
Amblyopia (%)	12 (± 6)	24	8	**.05**[c]	24	9	.08
First 5 years of follow-up							
No of patients = 41							
No of eyes = 77							
Secondary glaucoma (%)	25 (± 10)	26	24	.85	9	32	**.03**[c]
Glaucoma surgery (%)	30 (± 10)	35	28	.55	18	35	.14
Cataract surgery (%)	42 (± 11)	59	32	**.02**[c]	57	35	.08
Posterior synechiae (%)	40 (± 11)	56	33	.06	61	33	**.02**[c]
Band keratopathy (%)	16 (± 8)	22	12	.33	35	7	**.01**[c]
CME (%)	19 (± 9)	50	4	**<.01**[c]	41	12	**.01**[c]
Papillitis (%)	12 (± 7)	31	2	**<.01**[c]	23	8	.12
Strabismus (%)	4 (± 4)	9	3	.55	11	3	.21

CI = confidence interval; CME = cystoid macular edema; F = female; JIA = juvenile idiopathic arthritis; M = male; NA = not applicable.
[a]Additional exact frequencies of the complications are available at AJO.com in Supplemental Table 1.
[b]Within the first 1 year of follow-up no cases with glaucoma surgery were observed.
[c]Significant *P* values are highlighted in bold.

eyes from the initial uveitis group vs 29% of the eyes from the initial arthritis group; all $P \leq 0.04$). In contrast, secondary glaucoma was less common in the group with initial uveitis compared to the group with initial arthritis at 5 years of follow-up (9% vs 32%; $P = 0.03$; Table 2).

Multivariate analysis of prognostic factors for complications

No independent baseline risk factors for secondary glaucoma, strabismus, and amblyopia were found. Male gender appeared to be an independent risk factor for cataract surgery (adjusted hazard ratio [HR] = 4.33; 95% CI: 1.86–9.47; $P < 0.01$; Supplemental

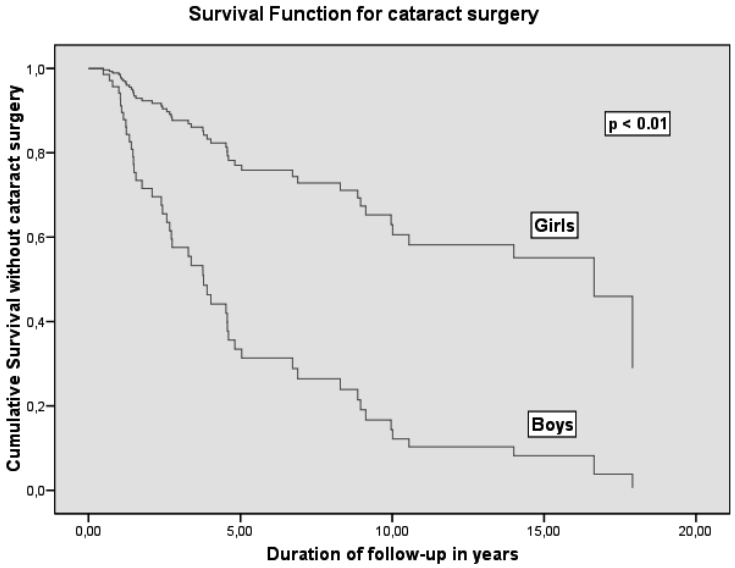
Figure 1), development of CME (HR = 4.59; 95% CI: 1.64–11.61; P = 0.01; Supplemental Figure 2), and papillitis (HR = 4.10; 95% CI: 1.21–13.44; P = 0.01; Supplemental Figure 3). Uveitis as initial JIA manifestation was only independently associated with development of posterior synechiae (HR = 3.21; 95% CI: 1.47– 6.36; P < 0.01; Supplemental Figure 4).

Discussion

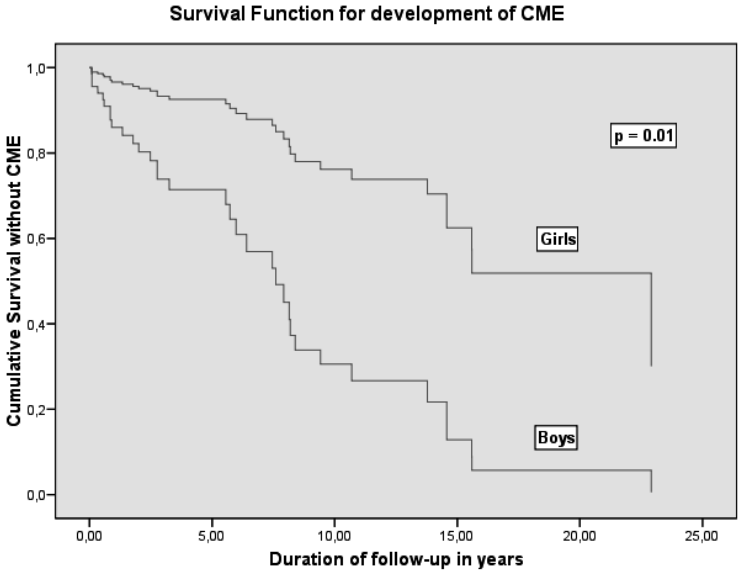
Our study shows that male gender is an independent baseline risk factor associated with multiple ocular complications of JIA-associated uveitis in our series. These findings are in accordance with earlier studies.[6;11–13] However, some of these studies found this correlation in univariate analysis only,[12] or in a heterogeneous study population.[6] Most of these studies investigated the cumulative complication rate or severity of uveitis but did not specify the complications.[11–13]

Development of CME, early cataract surgery, and papillitis were all independently associated with male gender. These findings imply that boys have more severe ocular disease, despite the fact that girls are at higher risk to be affected by anterior uveitis.[1;2;4] This observation is also surprising since male gender was not found to be a prognosticator of arthritis[21] and that some authors even found female gender to be associated with higher activity of arthritis and disability.[22;23] More severe uveitis in boys can possibly depend on some other gender-related host factors that could provoke more severe ocular inflammation. The independent association of male gender with increasedrisk for specific ocular complications of JIA-associated uveitis including CME, cataract, and papillitis has, to our knowledge, not been shown before. Children with uveitis prior to arthritis had more complications at various points of follow-up, with the exception of secondary glaucoma, which was less frequently observed. The majority of children initially presenting with arthritis are diagnosed earlier because of the screening programs, whereas children with initial uveitis can remain undetected because of the silent and insidious nature of JIA-associated uveitis.

The higher frequency of complications might hence be explained by a delay in effective treatment. However, although the children with initial uveitis had more and, often, multiple complications in univariate analysis, in multivariate analysis this turned out to be an independent risk factor only for posterior synechiae. The presence of posterior synechiae is known as a predictor for development of other complications, as reported previously.[13] The higher frequency of secondary glaucoma in the initial arthritis group



Supplemental figure 1. Survival plot for cataract surgery for boys' and girls' eyes with uveitis associated with juvenile idiopathic arthritis (JIA). Analysis is adjusted for age of uveitis onset, time between diagnosis of arthritis and uveitis, antinuclear antibody (ANA) serologic status, initial manifestation of JIA, presence of posterior synechiae at onset of uveitis, systemic administration of steroids, immunosuppression, and periocular injections. Boys versus girls hazard ratio (HR) = 4.33; 95% CI: 1.86–9.47; P < 0.01.



Supplemental figure 2. Survival plot for development of cystoid macular edema (CME) for boys' and girls' eyes with uveitis associated with juvenile idiopathic arthritis (JIA). Analysis is adjusted for age of uveitis onset, time between diagnosis of arthritis and uveitis, antinuclear antibody (ANA) serologic status, initial manifestation of JIA, presence of posterior synechiae at onset of uveitis, systemic administration of steroids, immunosuppression, and periocular injections. Boys versus girls hazard ratio (HR) = 4.59; 95% CI: 1.64–11.61; P = 0.01.

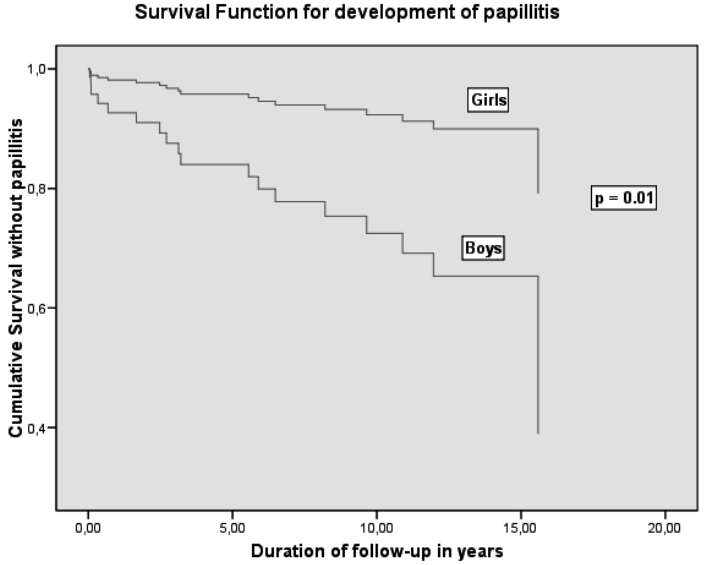
could be hypothetically explained by the fact that uveitis in children with classic presentation of JIA debuting with arthritis may receive treatment earlier and, therefore, reach remission earlier. It is well known that remissions can be associated with the rise of intraocular pressure and development of secondary glaucoma.[2] However, because of the limited size of the groups a spurious association cannot be excluded here.

Furthermore, we have observed a correlation between male gender and initial manifestation of JIA with uveitis instead of arthritis, which was not described before. Presentation with uveitis initially is noted previously in HLA-B27-positive boys.[24] However, in our cohort no difference in HLA-B27 status between boys and girls was observed within patients with initial uveitis. At this moment, there is no reasonable biological explanation for the association of male gender with atypical presentation of JIA and further evaluation is needed in larger series from other centers to confirm this observation.

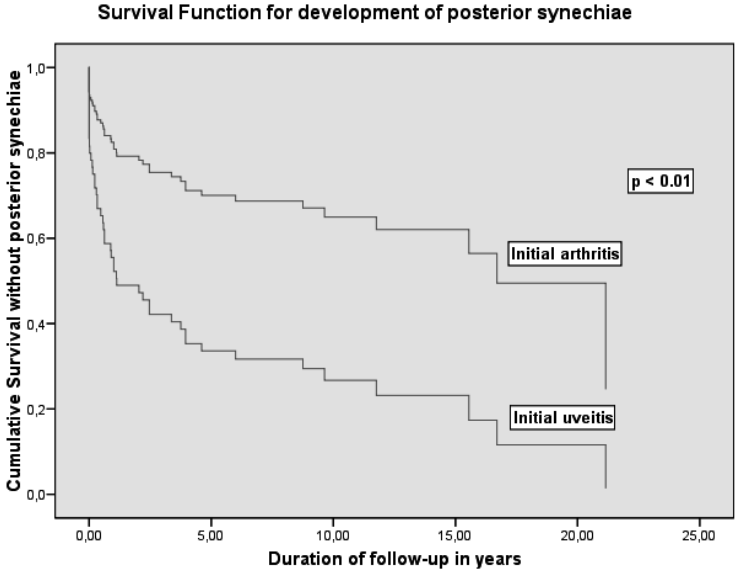
In summary, we showed worse prognosis in boys and an association of male gender with atypical manifestation of JIA in our series; nevertheless, additional analysis of complication rates within cases with exclusively classic manifestation of JIA (initial arthritis) showed still higher complication rates for cataract, CME, and papillitis in boys compared with girls.

Time interval between diagnosis of arthritis and uveitis was delineated by others as a risk factor for worse prognosis.[9–12] In most of these studies, the analysis of complications was not specific but cumulative. In our study the time interval was shorter in boys and in children from the younger-onset group, even after exclusion of cases with initial uveitis from the analysis. Adjusted for these and other potential confounders, the short interval between uveitis and arthritis was not significantly associated with complications in our series. So, the prognostic importance of the short interval between uveitis and arthritis for other complications could possibly be secondary to the effect of male gender.

Young age of onset of JIA-associated uveitis was not independently associated with poor prognosis or a severe course of uveitis. More frequent bilateral involvement in the younger age group was observed. A significantly shorter interval between arthritis and uveitis may indicate more active form of disease in young children. Several authors pointed out that young age of uveitis onset could be associated with poor prognosis;[6;9;12;15] however, these findings were not consistent.[7;16] In our series, adjusted for other factors, age of onset did not seem to play a significant role in prognosis of these patients. In any case, patients with young age of onset deserve additional attention because of the risk of amblyopia and strabismus.



Supplemental figure 3. Survival plot for development of papillitis for girls' and boys' eyes with uveitis associated with juvenile idiopathic arthritis (JIA). Analysis is adjusted for age of uveitis onset, time between diagnosis of arthritis and uveitis, antinuclear antibody (ANA) serologic status, initial manifestation of JIA, presence of posterior synechiae at onset of uveitis, systemic administration of steroids, immunosuppression, and periocular injections. Boys versus girls hazard ratio (HR) = 4.10; 95% CI: 1.21–13.44; P = 0.01.



Supplemental figure 4. Survival plot for posterior synechiae for eyes with uveitis as initial manifestation of juvenile idiopathic arthritis (JIA) versus eyes with uveitis secondary to arthritis. Analysis is adjusted for gender, age of uveitis onset, time between diagnosis of arthritis and uveitis, antinuclear antibody (ANA) serologic status, systemic administration of steroids, immunosuppression, and periocular injections. Initial uveitis versus initial arthritis hazard ratio (HR) = 3.21; 95% CI: 1.47–6.36; P < 0.01.

The current study has several notable limitations common to all retrospective studies. For instance, it was not possible to account for all possible confounders. Because our study originated in a tertiary center, the reported cumulative complication rate can be higher than would be found in secondary care centers where milder cases are treated. In addition, milder cases might have been lost at follow-up over time. The fact that boys in our series had a more complicated course of the disease and significantly longer follow-up supports this hypothesis. The possibility of an ascertainment bias should also be addressed. Because of the retrospective design, the time periods between visits can vary; thus it is possible that patients with more severe disease were seen more frequently, and therefore the likelihood of finding an ocular complication is higher in this group of patients. Another noteworthy limitation of our study is based on the limited number of patients (nevertheless, comparable with other studies in this field) and use of comparisons at multiple time points. This fact makes it more difficult to achieve statistical significance of potentially clinically significant data and increases the risk of type 2 error, although even with the current numbers of patients it was possible to show major differences in risk between genders.

For successful management of JIA-associated uveitis it is mandatory to identify the children who are at risk of complications and poor visual prognosis. Especially the definition of early prognostic factors, even present at the first visit to an ophthalmologist, could possibly improve the complication rates in JIA-associated uveitis. Our findings suggest that male gender may be associated with earlier development of vision-threatening complications and could be taken into prognostic consideration by those treating this group of patients.

SUPPLEMENTAL TABLE 1. Cumulative Incidences of Ocular Complications of Uveitis Associated With Juvenile Idiopathic Arthritis at Onset and 10 Years of Follow-up With 95% Confidence Intervals, According to Gender, and Initial Manifestation of Juvenile Idiopathic Arthritis

Complications	Total Percentage of Affected Eyes (± 95% CI)	Age of Uveitis Onset			Gender			Initial Manifestation of JIA		
		0–7 Years	7–16 Years	*P* Value	M	F	*P* Value	Uveitis	Arthritis	*P* Value
At onset										
No of patients = 65										
No of eyes = 117										
Secondary glaucoma (%)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Posterior synechiae (%)	10 (± 5)	13	4	.29	9	11	>.99	17	8	.18
Band keratopathy (%)	1 (± 2)	NA	4	.23	NA	1	>.99	NA	1	>.99
CME (%)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Papillitis (%)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Strabismus (%)	1 (± 2)	1	NA	>.99	NA	1	>.99	NA	1	>.99
Amblyopia (%)	13 (± 6)	15	NA	NC	19	10	.30	24	9	.12
First 10 years of follow-up										
No of patients = 22										
No of eyes = 43										
Secondary glaucoma (%)	42 (± 15)	46	30	.48	29	50	.18	44	41	.84
Glaucoma surgery (%)	35 (± 14)	41	20	.29	31	39	.64	33	37	.81
Cataract surgery (%)	65 (± 14)	73	40	.07	59	69	.52	69	63	.70
Posterior synechiae (%)	49 (± 15)	52	50	>.99	59	46	.41	75	36	.02
Band keratopathy (%)	28 (± 13)	21	50	.11	24	31	.74	44	19	.09
CME (%)	40 (± 15)	50	22	.25	63	30	.05	67	29	.02
Papillitis (%)	19 (± 12)	29	NA	.08	31	14	.24	33	13	.22
Strabismus (%)	14 (± 10)	21	NA	>.99	31	11	.21	33	11	.17

CI = confidence interval; CME = cystoid macular edema; F = female; JIA = juvenile idiopathic arthritis; M, male; NA = not applicable; NC = not comparable.

SUPPLEMENTAL TABLE 2. Cumulative Incidences of Ocular Complications in Children With Classic Manifestation of Uveitis Associated With Juvenile Idiopathic Arthritis After 1, 3, and 5 Years of Follow-up With 95% Confidence Intervals, According to Gender, and Initial Manifestation of Juvenile Idiopathic Arthritis Complications

Complications	Total Percentage of Affected Eyes (± 95% CI)	Gender		*P* Value
		M	F	
First 1 year of follow-up				
No of patients = 50				
No of eyes = 88				
Secondary glaucoma (%)	2 (±3)	NA	3	>.99
Cataract surgery (%)	7 (±5)	6	7	>.99
Posterior synechiae (%)	16 (±8)	22	15	.48
Band keratopathy (%)	2 (±3)	NA	3	>.99
CME (%)	7 (±5)	22	3	**.02**
Papillitis (%)	3 (±4)	17	NA	**.01**
Strabismus (%)	2 (±3)	3	NA	>.99
Amblyopia (%)	8 (±6)	12	8	.63
First 3 years of follow-up				
No of patients = 39				
No of eyes = 72				
Secondary glaucoma (%)	18 (±9)	25	16	.47
Glaucoma surgery (%)	8 (±6)	13	7	.61
Cataract surgery (%)	13 (±8)	38	5	**<.01**
Posterior synechiae (%)	21 (±9)	25	21	.74
Band keratopathy (%)	6 (±6)	13	4	.22
CME (%)	9 (±7)	31	4	**.01**
Papillitis (%)	6 (±6)	19	2	**.04**
Strabismus (%)	2 (±3)	7	NA	.24
Amblyopia (%)	8 (±6)	20	6	.13
First 5 years of follow-up				
No of patients = 29				
No of eyes = 54				
Secondary glaucoma (%)	31 (±12)	33	31	>.99
Glaucoma surgery (%)	35 (±13)	47	31	.35
Cataract surgery (%)	35 (±13)	47	31	.27
Posterior synechiae (%)	31 (±12)	40	30	.53
Band keratopathy (%)	7 (±7)	13	5	.31
CME (%)	11 (±8)	33	3	**.01**
Papillitis (%)	7 (±7)	27	NA	**.01**
Strabismus (%)	2 (±4)	8	NA	.30

CI = confidence interval; CME = cystoid macular edema; F = female; M = male; NA = not applicable.

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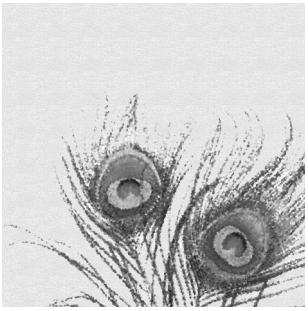

Chapter 4

Male gender and poor visual outcome in uveitis associated with juvenile idiopathic arthritis

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Abstract

Purpose: To analyze visual outcome in uveitis associated with juvenile idiopathic arthritis (JIA) according to age of onset of uveitis, gender, and initial manifestation of JIA.

Design: Retrospective nonrandomized interventional case series.

Methods: Visual outcome of 117 affected eyes (65 patients) with JIA-associated uveitis was noted at onset of uveitis and after 1, 3, and 5 years. Visual outcome was analyzed according to gender, age of onset of JIA-associated uveitis (<7 years and >7 years), and initial manifestation of JIA (as uveitis or as arthritis). Linear and logistic regression with generalized estimating equation (GEE) was performed.

Results: Median age of onset of uveitis was 4.2 years (range 1.5–16). Female-to-male ratio was 3:1. In 15 children (23%) uveitis was diagnosed before arthritis. Visual acuity of boys was significantly worse at 1 and 3 years of follow-up (both $P < 0.03$) but not at 5 years of follow-up ($P = 0.45$). Until 3 years after the diagnosis of uveitis, children with atypical initial manifestation of JIA (uveitis before arthritis) had significantly worse visual acuity compared with children in whom uveitis debuted after arthritis (all $P < 0.05$). No difference in vision between younger-onset (<7 years) and older-onset (>7 years) groups was noted. Blindness was independently associated with male gender (odds ratio [OR] = 6.61; 95% CI: 1.02–42.98; $P = 0.048$).

Conclusions: Male gender was an independent risk factor for poor visual prognosis in JIA-associated uveitis. Children in whom uveitis is being diagnosed before arthritis have significantly worse vision until 3 years after uveitis onset.

Introduction

Uveitis associated with juvenile idiopathic arthritis (JIA) is the most common form of uveitis in childhood.[1;2] JIA-associated uveitis typically has an asymptomatic, insidious onset and a potentially severe and complicated course. The complications during the course of JIA-associated uveitis can be sight-threatening.[2–5] The risk of amblyopia development attributable to vision-disturbing complications and media opacities deserves a special awareness in this group of young patients.[6]

Despite the current guidelines for the screening program and administration of new immunosuppressive agents, JIA-associated uveitis continues to be a potentially blinding condition. Although some centers report very good visual outcome,[7] some of the recent studies still show that up to 24% of patients end up blind or with severe visual impairment.[4;5;8;9]

Several authors contributed to the identification of early prognostic factors associated with poor visual outcome in JIA-associated uveitis. Although the results of the studies are not always conclusive, the following risk factors were pointed out as negative for visual outcome: young age of onset of uveitis,[4;10] severity of uveitis at first examination, [3;10;11] signs of vitreous involvement,[3] and short interval between the diagnosis of arthritis and uveitis.[11]

The prognostic role of gender in visual outcome of JIA-associated uveitis is still controversial.[3;10] In this study we evaluate the role of gender, age of onset of uveitis, initial manifestation of JIA, and other early prognostic factors for visual outcome in JIA-associated uveitis.

Patients and Methods

The same database as our other study on complications in JIA-associated uveitis.[12] The medical records of 65 pediatric patients (117 affected eyes) with uveitis associated with JIA or ANA-positive anterior uveitis were reviewed. The diagnosis of JIA was based on the criteria of the International League against Rheumatism.[13;14] All patients were examined by a pediatric rheumatologist who confirmed the diagnosis of JIA in 65 patients.

Evaluation of uveitis was made according to the criteria of the International Uveitis Study Group.[15] In addition to examination by an ophthalmologist who is specialized in childhood uveitis, all children in our clinics were being examined by an orthoptist

at the initial visit and regularly during the follow-up for screening of amblyopia. In this study amblyopia was scored only if a decrease in vision could not be clinically explained by vision-threatening complications of uveitis (media opacities and macular edema) at that moment.

In addition to the data gathered in our work on complications in JIA-associated uveitis,[12] best-corrected visual acuities (BCVAs) at standard points of time were assessed. Duration of uveitis was calculated from the date of diagnosis of uveitis.

The BCVAs were noted at the first presentation and after 1, 3, and 5 years of follow-up. We have categorized BCVAs according to definitions that are used as guidelines for obtaining a driver's license in various countries. These definitions are similar to definitions used by other studies: BCVA of 20/40 or better was defined as good, BCVA between 20/50 and 20/100 was defined as impaired, and BCVA of 20/200 or worse was defined as blindness.[3–5,7] Additionally we scored a visual field of less than 10 degrees as blindness.

We converted Snellen BCVAs into the logarithm of the minimal angle of resolution scale (logMAR) for statistical analysis and converted it back to Snellen BCVAs for data presentation.[16] In the analysis of visual outcome, only the eyes with vision loss attributable to uveitis or related complications were included.

Patients were subdivided according to their age at onset of uveitis, into a young-onset (uveitis onset < 7 years of age) or old-onset group (uveitis onset >7 years of age). This cut-off was chosen because of the higher likelihood of amblyopia developing before the age of 7. Two different types of initial manifestation of JIA were distinguished: initial manifestation as arthritis with later diagnosis of uveitis (classic); and initial manifestation with uveitis followed later by development of arthritis, making the diagnosis of JIA possible. Children in whom the signs of uveitis were present at the initial eye screening after the diagnosis of JIA were considered as classic presentation.

Statistical analysis of the data was performed with SPSS 15.0.1 (SPSS Inc, Chicago, Illinois, USA). We performed the analysis "by eye" including all affected eyes in the analysis. Correction for analysis of paired eyes was performed using generalized estimating equations (GEE).[17] However, analysis "by patient" was used in comparison of baseline constant host characteristics between subgroups: laterality, antinuclear antibody (ANA) status, administration of systemic therapy, duration of follow-up. The Pearson X^2 test or Fisher exact test was used for univariate analysis of categorical variables. LogMAR BCVAs at different points in time were compared using linear regression with GEE as adjustment for paired eyes in the analysis. Binary logistic regression with GEE was applied for multivariate analysis to identify independent pre-

dictive factors of blindness. In the multivariate analysis we entered baseline variables with $P \leq 0.05$ in univariate analysis and clinically significant potential confounders. Baseline variables that were associated with blindness in univariate analysis included gender, initial manifestation of JIA, and initial visual acuity. The model was additionally adjusted for age of onset of uveitis (as numeric variable) and duration of uveitis. P values of less than 0.05 were considered statistically significant. All significances were 2-tailed. In the presentation of the results we used the mean if the data were normally distributed (by Kolmogorov-Smirnov test; $P \leq 0.05$) and the median if not normally distributed.

Results

General characteristics of the study population

One hundred seventeen eyes (65 patients) were affected by JIA-associated uveitis, with available data of BCVA over a period longer than 1 year of follow-up. Table 1 presents general clinical characteristics of the whole study population with analysis of subgroups, according to age of onset of uveitis, gender, and initial manifestation of JIA. The typical manifestation of JIA-associated uveitis as chronic or recurrent anterior uveitis was observed in 116 eyes (99%), and in 1 eye (1%) panuveitis was present. Bilateral disease was more frequent in the younger-onset group: 43 of 48 patients (88%), versus 10 of 17 patients (59%) in the older-onset group ($P = 0.03$). Time between the diagnosis of arthritis and uveitis ranged from 0 to 12.3 years with a median of 0.7 years (Table 1). This interval was significantly shorter in the younger-onset group compared with the older-onset group and in boys compared with girls (both $P = 0.02$; Table 1). Children initially presenting with uveitis were less frequently ANA positive compared to children who presented with arthritis (73% vs 94%; $P = 0.05$). Follow-up was significantly longer in boys compared to girls (median 11.0 vs 7.2 years; $P = 0.05$).

Visual outcome

Median BCVAs at standard points of follow-up, analyzed according to the age of uveitis onset, gender, and initial manifestation of JIA, are presented in Table 2. Categorized BCVAs for the whole study population and the subgroups at standard points of follow-up are presented in Table 3.

Eleven eyes (11%; 11 patients) presented with BCVA of ≤ 20/200, meeting the criteria of legal blindness. The vision of 74 of 117 eyes (72%) of 42 patients was better than

TABLE 1. General Characteristics of Patients With Uveitis Associated With Juvenile Idiopathic Arthritis According to Age of Uveitis Onset Gender, and Initial Manifestation of Juvenile Idiopathic Arthritis

	Age of Uveitis Onset			Gender			Initial Manifestation of JIA		
Characteristics	0–7 Years N = 48 (%)	7–16 Years N = 17 (%)	*P* Value	M N = 18 (%)	F N = 47 (%)	*P* Value	Uveitis N = 15 (%)	Arthritis N = 50 (%)	*P* Value
Female subjects, n (%)	35 (73)	12 (71)	>.99	NA	47 (100)	NC	7 (47)	40 (80)	**.02**[b]
Age of onset, median (range)	NC	NC	NC	4.3 (1.7–13.8)	4.2 (1.7–16.0)	.48	4.0 (1.7–12.1)	4.5 (1.5–16.0)	.68
Bilateral (%)	42 (88)	10 (59)	**.03**[b]	16 (89)	36 (77)	.33	14 (93)	38 (76)	.27
Time between diagnosis of arthritis and uveitis,[a] median (range)	0.7 (0–6.7)	2.0 (0–12.3)	**.02**[b]	0.3 (0–5.0)	1.0 (0–12.3)	**.02**[b]	0.3 (0.1–7.3)	0.8 (0–12.3)	.86
Uveitis as initial manifestation of JIA	12 (25)	3 (18)	.74	8 (44)	7 (15)	**.02**[b]	15 (100)	NA	NC
Follow-up,[a] median (range)	8.3 (1.1–27.5)	7.2 (1.2–23.1)	.51	11.0 (1.1–27.5)	7.2 (1.1–22.9)	**.05**[b]	12.6 (1.1–27.5)	6.1 (1.1–23.1)	.07
ANA-seropositive	42 (91)	13 (81)	.36	16 (94)	39 (87)	.66	11 (73)	44 (94)	**.05**[b]
Systemic corticosteroids	18 (38)	8 (47)	.53	8 (44)	18 (39)	.78	10 (67)	16 (33)	**.02**[b]
Immunomodulatory therapy	39 (81)	12 (71)	.49	15 (83)	36 (77)	.74	12 (80)	39 (78)	>.99

F = female; JIA = juvenile idiopathic arthritis; M = male; NA = not applicable; NC = not comparable.
[a]Time is presented in years.
[b]Significant *P* values are highlighted in bold.

or equal to 20/40 at presentation (Table 3). Median decimal fraction of BCVA for the whole group at onset was 0.8 (Table 2). Children who presented initially with uveitis had significantly worse median BCVA at onset compared with children in whom uveitis developed after the arthritis (decimal fraction BCVA 0.4 vs 0.9; P < 0.01). At onset, BCVA of ≤ 20/200 was observed in 25% of eyes in the initial uveitis group compared with 5% of the initial arthritis group (P < 0.01; Table 3). Visual acuity of ≥ 20/40 at onset was present in only 43% of the affected eyes from the initial uveitis group, in contrast with 83% of eyes from the initial arthritis group (P < 0.01; Table 3). At 1 year of follow-up the median decimal fraction BCVA for the whole study population was 1.0, but visual outcome of the initial uveitis group was still significantly worse compared to the initial arthritis group (median decimal fraction BCVA 0.7 vs 1.0 respectively; P = 0.01; Table 2). The initial uveitis group had 23% of legally blind eyes versus 4% in the initial arthritis group at 1 year of follow-up (P = 0.02; Table 3). The difference between median BCVAs of boys and girls at 1 year of follow-up was significant (0.9 vs 1.0; P = 0.02). At 1 year of follow-up 22% of boys' eyes were legally blind and only 66% had good visual acuity, versus 3% and 89%, respectively, of girls' eyes (P = 0.01; Table 3). At 3 years of follow-up visual outcome of boys and children with initial uveitis was significantly worse (Table 2). Median decimal BCVA of boys at 3 years of uveitis follow-up was 0.5, compared with 1.0 in girls (P = 0.03; Table 2).

No significant differences in BCVA at standard points of follow-up between groups with younger and older onset (<7 and >7 years) were delineated (Table 3). Additional analysis of visual outcome of children with uveitis onset < 3 years was performed, but the result did not differ from the previous analysis.

TABLE 2. Long-Term Visual Acuities in Eyes Affected by Uveitis Associated With Juvenile Idiopathic Arthritis, According to Age of Uveitis Onset, Gender, and Initial Juvenile Idiopathic Arthritis Manifestation

		Age of Uveitis Onset			Gender			Initial Manifestation of JIA		
BCVA	Total	0–7 Years	7–16 Years	*P* Value[b]	M	F	*P* Value[b]	Uveitis	Arthritis	*P* Value[b]
At onset										
No of eyes/patients = 108/59										
Median[a]	0.8	0.8	0.8	.66	0.6	0.8	.06	0.4	0.9	**<.01**[c]
1 year of follow-up										
No of eyes/patients = 101/56										
Median[a]	1.0	1.0	1.0	.27	0.9	1.0	**.02**[c]	0.7	1.0	**.01**[c]
3 years of follow-up										
No of eyes/patients = 79/43										
Median[a]	0.8	0.8	1.0	.07	0.5	1.0	**.03**[c]	0.5	1.0	**.05**[c]
5 years of follow-up										
No of eyes/patients = 60/33										
Median[a]	0.7	0.7	0.6	.50	0.6	0.7	.45	0.6	0.8	.67

BCVA = best-corrected visual acuity; F = female; JIA = juvenile idiopathic arthritis; M = male.

[a]To compute the median of VAs all decimal Snellen BCVAs were transformed to their logMAR equivalents and afterwards back to decimal BCVAs, which are presented.

[b]*P* values are adjusted for the usage of paired eyes in the analysis.

[c]Significant *P* values are highlighted in bold.

TABLE 3. Long-Term Categorized Visual Outcomes in Eyes Affected by Uveitis Associated With Juvenile Idiopathic Arthritis, According to Age of Uveitis Onset, Gender, and Initial Juvenile Idiopathic Arthritis Manifestation

	Total Number of Affected Eyes	Age of Uveitis Onset		*P*	Gender		*P*	Initial Manifestation of JIA		*P*
BCVA	(%± 95% CI)	0–7 Years	7–16 Years	Value[a]	M	F	Value[a]	Uveitis	Arthritis	Value[a]
At onset										
Number of eyes = 103										
Number of patients = 59										
≤20/200 (%)	11 (11 ± 5.7)	10 (13)	1 (4)	.31	6 (18)	5 (7)	.11	7 (25)	4 (5)	**<.01**[b]
20/100–20/50 (%)	18 (17 ± 7.4)	12 (15)	6 (25)		8 (24)	10 (15)		9 (32)	9 (12)	
≥20/40 (%)	74 (72 ± 8.6)	57 (72)	17 (71)		20 (59)	54 (78)		12 (43)	62 (83)	
1 year of follow-up										
Number of eyes = 95										
Number of patients = 56										
≤20/200 (%)	9 (10 ± 5.9)	8 (11)	1 (4)	.60	7 (22)	2 (3)	**.01**[b]	6 (23)	3 (4)	**.02**[b]
20/100–20/50 (%)	9 (10 ± 6.1)	6 (8)	3 (13)		4 (13)	5 (8)		3 (12)	6 (9)	
≥20/40 (%)	77 (80 ± 7.9)	58 (81)	19 (83)		21 (66)	56 (89)		17 (65)	60 (87)	
3 years of follow-up										
Number of eyes = 78										
Number of patients = 43										
≤20/200 (%)	3 (4 ± 4.3)	3 (5)	0 (0)	.51	2 (7)	1 (2)	**.01**[b]	2 (11)	1 (2)	**.06**[b]
20/100–20/50 (%)	6 (8 ± 6.0)	6 (10)	0 (0)		5 (19)	1 (2)		3 (16)	3 (5)	
≥20/40 (%)	69 (89 ± 6.9)	53 (86)	16 (100)		20 (74)	49 (96)		14 (74)	55 (93)	
5 years of follow-up										
Number of eyes = 58										
Number of patients = 33										
≤20/200 (%)	4 (7 ± 6.5)	3 (7)	1 (8)	>.99	0 (0)	4 (10)	.37	0 (0)	4 (9)	.68
20/100–20/50 (%)	10 (17 ± 9.5)	8 (18)	2 (15)		4 (21)	6 (15)		3 (19)	7 (17)	
≥20/40(%)	44 (76 ± 10.7)	34 (76)	10 (77)		15 (79)	29 (74)		13 (81)	31 (74)	

BCVA = best-corrected visual acuity; F = female; JIA = juvenile idiopathic arthritis; M = male.

[a]Analysis performed "by eye" with adjustment for paired eyes.

[b]Significant *P* values are highlighted in bold.

Amblyopia

Eleven patients in our series (17%) presented with amblyopia attributable to uveitis at their first visit to an ophthalmologist. Three more patients developed amblyopia in the second year of follow-up. Cumulatively, amblyopia was present in 14 of 65 patients (22%). Cataract (n = 7) and long-term administration of atropine eye drops (n = 2) were the most frequent causes of amblyopia, and in the 5 other patients there was a combination of causes. There were no new cases of amblyopia after 2 years of follow-up. At 3 years of follow-up there were significantly more boys' eyes (24%) with amblyopia compared to girls' eyes (8%; P = 0.05).

Visual loss and blindness

During the follow-up, 12 blind eyes in 10 patients were identified. Table 4 provides clinical characteristics of these patients and causes of their blindness. Boys' eyes (7/34; 21%) were significantly more frequently affected by blindness than girls' eyes (5/83; 6%; P = 0.04). Furthermore, blindness was bilateral in 2 male patients. The majority of patients with blind eyes had initial uveitis instead of arthritis (58% vs 42%; P = 0.01). Secondary glaucoma was the most common cause of visual loss (6 of 12 eyes; 50%). Other causes of blindness were related to cataract surgery with development of cyclitic membranes (4 of 12 eyes; 33%) and cystoid macular edema (CME) (3/13; 25%) (Table 4). In 4 of 12 eyes (33%) amblyopia played a role in development of definitive loss of vision.

Our series contained 7 patients (13 eyes) with diagnosis of uveitis made before 1990. There were 3 blind eyes in this group (3/13; 23%) compared with 9 of 104 (11%), but this difference was not statistically significant (P = 0.13).

TABLE 4. Characteristics of Blind Eyes in Patients With Uveitis Associated With Juvenile Idiopathic Arthritis

Number of Blind Eye	Gender	Age of Onset of Uveitis (Years)	Initial Manifestation of JIA	BCVA ≤ 20/200	Visual Field < 10 Degrees	Duration of Uveitis Before Blindness (Years)	Cause of Blindness
1[a]	M	3.9	Uveitis	yes	yes	1.2	Complicated cataract extraction, amblyopia
2[a]				yes	no	12.9	Complicated cataract extraction
3[b]	M	5.1	Arthritis	no	yes	14.3	Secondary glaucoma
4[b]		6.4		yes	yes	3.3	Secondary glaucoma
5	M	3.0	Uveitis	yes	yes	4.8	Secondary glaucoma
6	M	4.4	Uveitis	yes	no	9.6	Secondary glaucoma
7	M	4.0	Uveitis	yes	no	11.7	Amblyopia, CME
8	F	2.6	Arthritis	yes	no	0.7	Complicated cataract extraction, amblyopia
9	F	3.0	Arthritis	yes	no	4.5	Amblyopia, CME
10	F	3.7	Uveitis	yes	yes	6.9	Secondary glaucoma
11	F	12.6	Arthritis	yes	yes	2.0	Secondary glaucoma
12	F	12.1	Uveitis	yes	no	6.5	Complicated cataract extraction, CME

BCVA = best-corrected visual acuity; CME = cystoid macular edema; F = female; JIA = juvenile idiopathic arthritis; M = male.
[a,b]Fellow eyes.

Multivariate analysis of prognostic factors for blindness

Baseline factors associated with blindness in univariate analysis included initial uveitis (P = 0.01), male gender (P = 0.04), and visual acuity at the first visit to ophthalmologist (P = 0.02). When we entered these factors together with age of uveitis onset and duration of uveitis (both as continuous variables) to the binary logistic regression model with adjustment for paired eyes, male gender appeared to be the only significant independent risk factor for blindness (odds ratio [OR] = 6.61; 95% CI: 1.02– 42.98; P = 0.048).

Discussion

This study demonstrates a trend toward worse visual outcome in boys with JIA-associated uveitis. Furthermore, in our series male gender was an independent factor associated with blindness in JIA-associated uveitis. The unfavorable prognostic role of male gender has been previously suggested by several authors; however, these studies mainly showed the effect of the male gender on the development of complications in the course of JIA-associated uveitis.[18–20] Recent results of Holland and associates showed that male gender was unfavorable for visual outcome as well and, similar to our results, male gender was found to be independently associated with blindness.[3] These analogous findings are also noteworthy because Holland and associates included a more heterogeneous study population, which included all cases of chronic anterior uveitis, with JIA patients being a minority. Therefore, male gender was identified as a risk factor for blindness in anterior uveitis in children independently of the underlying diagnosis. These findings might imply that boys have more severe ocular disease, despite the fact that girls are at higher risk to be affected by this form of uveitis.[2,7]

In contrast, an earlier study by Dana and associates showed that male gender was associated with visual improvement over time and that girls had worse visual outcome.[10] The reason for the observed differences is not clear, but they might be explained by conspicuous baseline differences between our study populations, including the relatively high mean age of onset of JIA-associated uveitis in Dana and associates' study compared to our data.

Poor visual prognosis in boys could probably be explained by an association of male gender with a complicated course of JIA-associated uveitis.[18–20] Also, in our article on complications in JIA-associated uveitis,[12] we have concluded that male gender is independently associated with development of sight-threatening complications, namely

CME, cataract, and papillitis. These are important causes of visual acuity loss in this population, as shown earlier and validated in the present study.[3–5,7] These complications can potentially lead to development of amblyopia in children of a young age and contribute to an even worse visual outcome. In our series, boys developed amblyopia significantly more frequently, which can also contribute to their lower vision.

Although the possible negative role of young age at onset of disease for visual outcome has been suggested by some authors,[4,10] these findings were not consistent.[21] Young age of onset might be associated with amblyopia, and therefore these patients deserve special attention and regular orthoptic evaluations. Amblyopia complicated more than 20% of cases of JIA-associated uveitis in the present study, and might contribute to the severe loss of vision in young patients. It should be mentioned that the diagnosis of amblyopia is difficult in this group of patients with uveitis and its complications. Sometimes it is impossible to distinguish amblyopia from damage caused by complications of uveitis. In this study amblyopia was diagnosed only if a decrease in vision could not be declared by the presence of vision-threatening complications of uveitis, such as cataract or CME.

We found that children with initial uveitis present with worse visual acuity and maintain a worse visual outcome up to 3 years of follow-up. This is not very surprising, since the worse visual outcome can be the result of more severe uveitis or a prolonged presence of untreated disease. In the last case, many sight-threatening complications can be present at the time of diagnosis. However, in the multivariate analysis, initial uveitis was not independently associated with blindness and its effect is most likely inferior to the effect of male gender due to the correlation between these 2 variables, which is further addressed in our article on complications in JIA-associated uveitis.[12]

There are several limitations attributable to the retrospective design of the present study, which makes it impossible to account for all possible confounders and to estimate exactly the causal associations. Changes in clinical approach over the 2½ decades are unavoidable and are not easy to account for. Because our study was originated in a tertiary center, the reported outcome can possibly be worse than in milder cases treated in secondary care centers. One can also assume that milder cases have shorter follow-up in our center. It is an interesting fact that in our series, boys had significantly longer follow-up than girls, which can indirectly indicate a more severe course of disease in boys. Despite the mentioned limitations, our study was able to detect a substantial difference in visual outcome between boys and girls.

The majority of children in our study showed general improvement of vision during the follow-up. This was probably attributable to the higher awareness, the screening

program, and new, aggressive therapies.[22] The treatment of JIA-associated uveitis has changed over the last 20 years. Whether children with diagnosis of uveitis made earlier than 2 decades ago have worse visual outcome than children who were diagnosed more recently cannot be concluded from our study because of the relatively small number of patients diagnosed before 1990. Despite the achievements of the past years, a considerable percentage of eyes (10% in our study) still ended up with loss of vision. This fact remains a great concern for all those treating these patients. Our findings suggest an association between male gender and poor visual prognosis in JIA-associated uveitis. At this moment it is unlikely that this observation will change the clinical approach to an individual patient. Nevertheless, it remains mandatory to identify children who are at high risk of sight-threatening complications by early screening with attention to possible baseline risk factors.[3–5;7;18–20]

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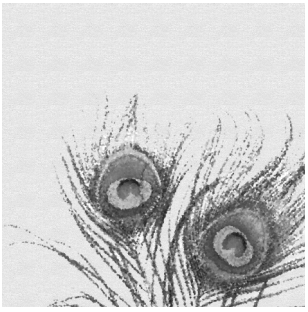

Chapter 5

The clinical course of juvenile idiopathic arthritis associated uveitis in childhood and puberty

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Abstract

Aim: The long-term course of juvenile idiopathic arthritis (JIA)-associated uveitis is not known yet. This study investigates the course and activity of JIA-associated uveitis in childhood and puberty.

Design: Retrospective study of the clinical data of 62 JIA patients with uveitis. The main outcome measurements consisted of uveitis activity measured as mean cell grade in the anterior chamber, topical and systemic medication and ocular complications related to disease activity. All data were scored and evaluated per year of age.

Results: Uveitis activity took a biphasic course with a quiet phase around the age of 9 years and showed increased activity during early teenage years. The biphasic course was significantly related to age ($p = 0.048$) but not to uveitis duration. More patients were treated with systemic immunosuppressive medication in estimated puberty years (63% in boys,53% in girls) compared with prepuberty years (46% and 28%, respectively), although the difference was only significant in girls ($p < 0.001$). The presence of cystoid macular oedema and papillitis was not significantly related to estimated puberty, but the development of an hypotonous eye was more frequently observed in boys in estimated puberty years ($p = 0.026$).

Conclusions: JIA-associated uveitis appears to take a biphasic course with the second phase of activity during early teenage years and more treatment with systemic immunosuppressive medication occurred during estimated puberty compared with prepuberty years.

Introduction

Uveitis is an extra-articular manifestation of juvenile idiopathic arthritis (JIA) of which the course of disease and required treatment is not exactly known. Uveitis occurs in about 12-13% of patients with all types of JIA.[1;2] Several studies investigated the complication rates and visual outcomes in older children,[1;3;4] but there is little known about the long-term course of the uveitis activity. In one study investigating disease course of JIA, half of the oligoarticular JIA patients reached remission of arthritis at age 16.5 However, although arthritis and uveitis are manifestations of one systemic disease, the flare-ups of arthritis and ocular inflammation occur independent of each other.[6]

It is remarkable that although girls with JIA are known to have a higher risk of developing uveitis, boys with uveitis appear to have a more serious progression of disease, with a poorer prognosis.[7-9] The cause of these differences between genders is unknown but it might suggest involvement of hormonal influence.

The objective of this study is to investigate the course of uveitis progression over time, and specifically to determine the activity of uveitis, ocular complications and medical treatment in children with uveitis associated with JIA before, during and after puberty.

Methods

Clinical data of 62 patients with JIA-associated uveitis, who were examined at the University Medical Centre at Utrecht, Groningen or Leiden between 1980 and 2010 were studied.

To be included in the study, patients had to be diagnosed at least 1 year before estimated onset of puberty and follow-up should have lasted at least 1 year after the estimated onset of puberty. Based on the results of a study of pubertal development in The Netherlands, in girls puberty was estimated to last from their 10th up until their 15th birthday, and in boys from the age of 11 until their 16th birthday.[10;11]

In all cases, the diagnosis of JIA was made in agreement with the criteria from the International League against Rheumatism by a paediatric rheumatologist.[12] Diagnosis of uveitis was made in agreement with the International Uveitis Study Group recommendations.[13] For every patient, the following data were recorded: gender, date of birth, age at onset of uveitis and age at onset of JIA.

For every year of age during follow-up, the following data were collected: percentage of time with active uveitis, average cell grade during activity, presence of complica-

tions related to active uveitis; cystoid macular oedema, papillitis and hypotony. A period of activity was assumed to last from the first visit with active uveitis after a period of inactivity to the next visit without active uveitis. Patients were seen at least six times per year. Active uveitis was defined as at least 1+ cells in the anterior chamber, using the grading system advocated by the Standardization of Uveitis Nomenclature working group.[14] Average cell grade per year was calculated by multiplying the percentage of time of activity by the cell grade score during activity. Hypotony was defined as an intraocular pressure of <6 mm Hg in at least two consecutive visits. Other data recorded per year of age were: average frequency of daily topical corticosteroid administration (converted to the equivalent dosage in prednisolon 1%), systemic immunosup-

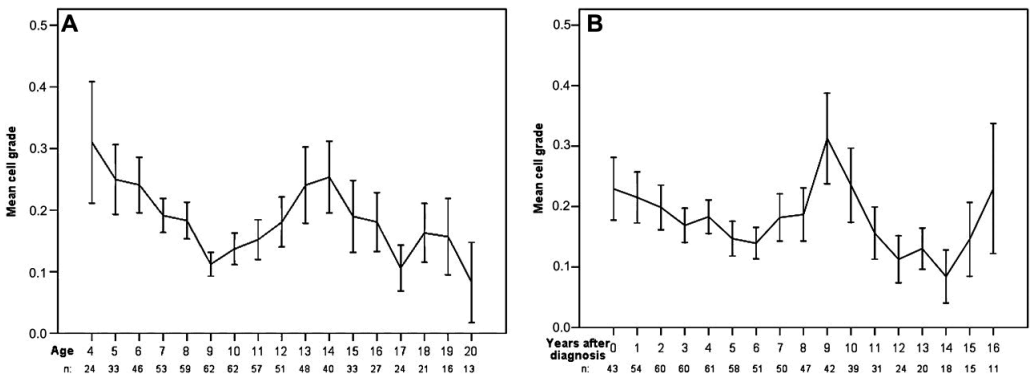

Figure 1. Course of uveitis activity expressed by a mean cell grade in the anterior chamber in children with juvenile idiopathic arthritis-associated uveitis according to age and duration of disease. (A) Mean cell grade per year of age and (B) for every year after diagnosis. n=number of patients included at that age. Error bars: ±SE.

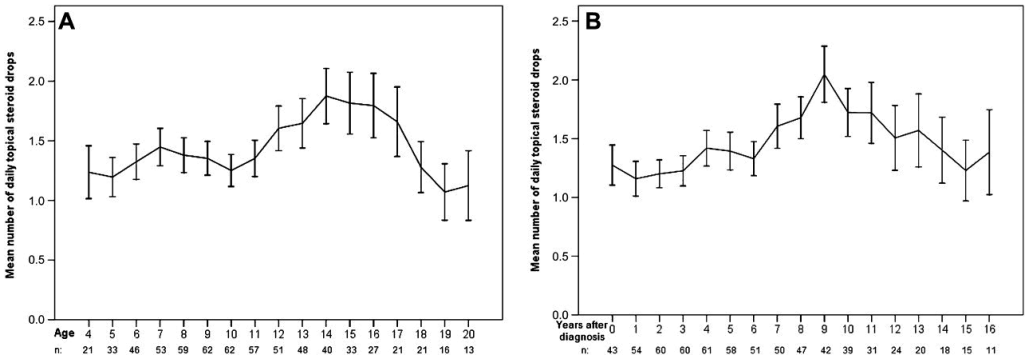

Figure 2. Course of daily topical corticosteroid therapy in children with juvenile idiopathic arthritis-associated uveitis according to age and duration of disease. (A) Average number of daily eye drops per year of age and (B) for every year after diagnosis of uveitis. n=number of patients included at that age. Error bars: ±SE.

pressive medication used for more than 6 months per year, including methotrexate, prednisone, anti-tumour necrosis factor (TNF)a agents and other immunosuppressive medications (mycofenolate mofetil, cyclosporine or azathioprine). Methotrexate starting dose was 10-15 mg/m2 bodysurface once a week, with a maximum dosage of 20 mg/m2. Generally, the starting dose of oral prednisone was 1 mg/kg bodyweight.
When data on therapy compliance were available, medication reported as used by patient or parent was recorded; otherwise, medication was assumed to be used as prescribed. Uveitis activity, complications and medication prescribed within 2 months of and directly related to intervening operations were not included. According to the estimated puberty criteria mentioned above, all available patient years were divided into prepuberty and estimated puberty years.
The database was built by patient, including the worst inflamed eye at every visit. Statistical analysis was performed using SPSS V.15.0.1 software (SPSS Inc.). A X^2 test was used for the analysis of categorical values, whereas the ManneWhitney U test was performed on continuous variables that were not normally distributed. Bonferroni correc-

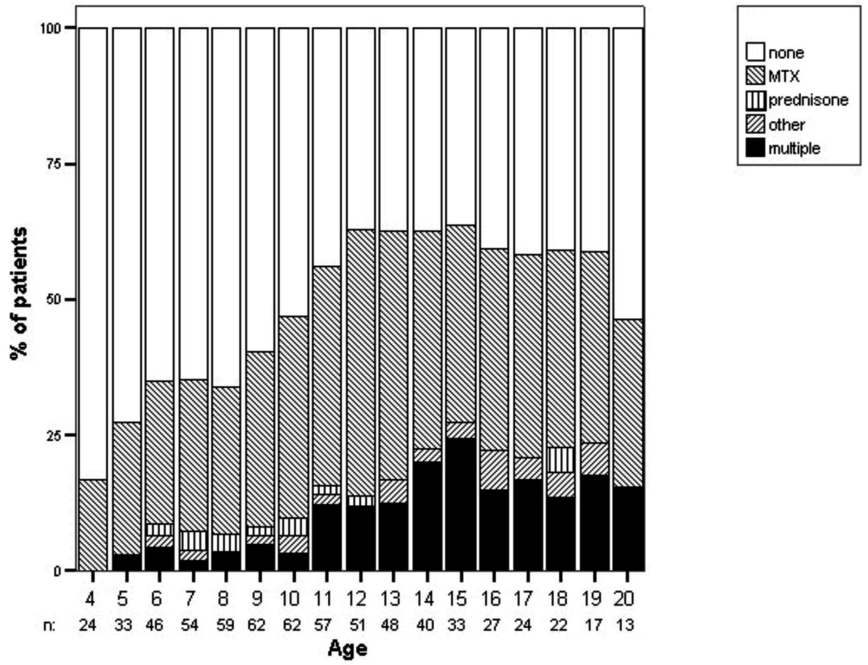

Figure 3. Use of systemic medication over time in children with juvenile idiopathic arthritis-associated uveitis. MTX, methotrexate. Other medications included mycofenolate mofetil, cyclosporine or azathioprine. Anti-TNFa medications were in all cases prescribed as a part of combination therapy and therefore all cases were classified into the category 'multiple'. N=number of all patients included in the specific age category.

tion was applied in case of multiple testing. Repeated measurement analysis was used for the analysis of time progression of continuous variables. Repeated measurement analysis was performed only including patients with at least 9 years of follow-up data available, encompassing at least 4 years before and 4 years during estimated puberty. With the above mentioned puberty definitions, for boys and girls together, this included 29 patients with data available for ages 6-14. In order to try to differentiate between the influence of puberty and the influence of the long-term course of uveitis, the same calculation was performed for duration of uveitis, including the 35 patients who had data available from 1 to 9 years after diagnosis of uveitis. Means were reported for normally and medians for not normally distributed variables. P values ≤ 0.05 were considered significant.

Results

Study population

General characteristics of the study population are displayed in table 1. Girls were significantly younger than boys at diagnosis of JIA; however, the age at diagnosis of JIA-associated uveitis was similar for boys and girls. Therefore, uveitis in boys was more frequently diagnosed before the onset of arthritis.

Uveitis activity

Figure 1 represents changes in mean cell grade over time, per year of age (figure 1A) and per year after diagnosis of uveitis (figure 1B). In general, a high cell grade was found during early years of age (4e6 years of age), followed by a quiet stage around the age of 9 and 10, after which activity rose again. Repeated measurement analysis including the 29 patients with available data over all ages from 6 to 14 showed a significant association between the age of patients and the mean cell grade values (p =

Table 1 General characteristics of patients with JIA-associated uveitis

Characteristics	Total	Boys	Girls	p Value
Number of patients	62	22	40	NA
Mean age (years) at diagnosis of uveitis (±SD)	4.9 (±1.7)	5.0 (1.7)	4.8 (1.8)	0.762
Median age (years) at diagnosis of JIA (range)	3.5 (1.0–11.2)	4.5 (2.9–9.1)	3.1 (1.0–11.2)	**<0.001**
JIA before uveitis (%)	49 (79)	11 (50)	38 (95)	**<0.001**
Mean follow-up in years (±SD)	11.4 (±4.3)	11.4 (4.1)	11.3 (4.3)	0.910

p Values ≤0.05 are highlighted in bold.
JIA, juvenile idiopathic arthritis; NA, not applicable.

0.048). This association was significantly compatible with a quadratic model (p=0.018), whereas other models (linear, cubic and higher orders) did not fit with our data (p = 0.815, 0.980 and 0.103-0.249, respectively). The compatibility to a quadratic model indicates that over the ages 6-14, the average cell grade in the anterior chamber takes a U-shaped course, as is seen in figure 1A.
The fluctuations of mean cell grade over the period of 1-9 years after diagnosis of uveitis (35 patients) were not significant (p = 0.300). There were no significant differences in cell grade patterns between boys and girls, or between patients with onset of uveitis first and arthritis first.

Medication

Figure 2 shows changes in daily topical corticosteroid therapy over time for age (figure 2A) and for number of years after diagnosis of uveitis (figure 2B). The mean frequency of daily topical corticosteroid use at ages 6e14 did not show any significant changes over time (p = 0.314). The frequency of daily corticosteroid drops did show a significant association to disease duration in the period of 1-9 years after diagnosis of uveitis (p = 0.003). This association fits a linear model (p = 0.02) and was not significantly compatible with other models (quadratic, cubic or higher orders, respectively, p = 0.345, 0.109 and 0.054-0.856). This linear association of number of daily steroid drops to timein the period of 1-9 years after diagnosis appears to be a linear increase, as is seen in figure 2B.
There were no significant differences in topical corticosteroid patterns between boys and girls, or between patients with onset of uveitis first and arthritis first.
Treatment with systemic immunosuppressive and/or corticosteroid medication per year of age is shown in figure 3. Anti-TNFa medication was always prescribed in combination with methotrexate, causing the 13 patients (21%) who ever used anti-TNFa agents to be in the 'multiple' category.
The chart for years after the diagnosis of uveitis revealed a similar pattern (data not shown). The use of systemic immunosuppressive therapy is indicated in table 2.
Systemic immunosuppressive medication was significantly more often administered during the estimated puberty years than in the prepuberty years in girls (p < 0.001). In boys, systemic immunosuppressive medication was also increased in estimated puberty years; however, this was no longer significant after Bonferroni correction (p = 0.014 before correction, p = 0.112 after correction). Of the children diagnosed with uveitis between 1990 and 1994, 23% started systemic medication within 5 years, whereas this number has increased to 78% in patients diagnosed in 2000-2004. To find out if the

increase in therapy at later ages was the result of more aggressive treatment strategies after the turn of the century, we also compared systemic immunosuppressive treatment in prepuberty versus estimated puberty years only including patients diagnosed during or after the year 2000. These girls used systemic treatment in 25 of 64 (39%) prepuberty years compared with 24 of 37 estimated puberty years (65%) (p = 0.012). Boys diagnosed with uveitis since 2000 used systemic treatment in 24 of 36 (67%) prepuberty years, compared with 19 of 22 (86%) puberty years (p = 0.097).

Complications

Uveitis activity-related complications in prepuberty and estimated puberty years are displayed in table 2. The development of complications in the prepuberty and estimated puberty years did not differ with the exception of hypotony, which was more frequently observed in boys during estimated puberty compared with prepuberty years (p = 0.003). Eleven patients had hypotony at any time. Five were directly related to uveitis activity. Two cases were at least 2 months after, but possibly related to glaucoma implant surgery and the remaining three were likely caused by ciliary body insufficiency after long-term course of uveitis.

Table 2 Systemic treatment and activity related complications in prepuberty versus estimated puberty years in children with juvenile idiopathic arthritis-associated uveitis

	Total patient years	Prepuberty years, positive prepuberty years/total prepuberty years (%)	Estimated puberty years, positive estimated puberty years/total estimated puberty years (%)	p Value
Systemic medication				
Boys	211	56/121 (46)	57/90 (63)	0.112
Girls	345	52/187 (28)	84/159 (53)	**<0.001**
CMO				
Boys	200	30/115 (26)	22/85 (26)	1.000
Girls	328	15/177 (8)	19/151 (13)	1.000
Papillitis				
Boys	200	18/115 (16)	7/85 (8)	0.935
Girls	328	13/177 (7)	20/151 (13)	0.613
Hypotony				
Boys	211	2/121 (2)	10/90 (11)	**0.026**
Girls	339	6/183 (3)	4/156 (3)	1.000

Years positive for systemic immunosuppressives and/or corticosteroid medication indicate medication used for at least half of that year. Years positive for CMO (cystoid macular oedema) or papillitis indicate years in which that complication was observed for at least one visit, For hypotony, years with two consecutive visits of intraocular pressure <6 mm Hg were counted as positive. Total patient years differ between complications and systemic medication because of missing data.
Estimated puberty years in girls: ages 10–14, in boys: ages 11–15.
Bonferroni correction was applied.
p Values ≤0.05 are highlighted in bold.

Discussion

The results of this study suggest that JIA-associated uveitis encompasses a biphasic course; a high initial disease activity, followed by a quiet stage and a new wave of activity during early teenage years. This increase in uveitis activity during early teenage years was also indirectly reflected by the increased use of immunosuppressive medication in estimated puberty years. After Bonferroni correction, the difference between systemic treatment in prepuberty versus estimated puberty years was no longer significant in boys. This might be caused by more treatment in prepuberty years in boys, which is possibly related to a more severe and complicated course of uveitis in boys.[9;15;16]

The increase in systemic immunosuppressive treatment at older ages might also be the result of increasingly aggressive treatment strategies over the years. This makes calendar year an important confounder. Because we found an increase in subscribing systemic therapy until the year 2000, we redid the analysis only including patients diagnosed since then. This analysis showed the same trend, which makes it unlikely thatthe increase was caused by more aggressive treatment strategies alone.

The reimbursement of anti-TNFa agents was established in The Netherlands in August 2007, after which all patients had access to anti-TNFa agents regardless of financial status. There is a lack of data on long-term course of uveitis in JIA but it has been found that at re-evaluation in adulthood (a mean of 19.3 years after diagnosis of JIA), 42% of patients still had active uveitis.[17]

We attempted to distinguish the effect of puberty from the straightforward association with the duration of ocular disease, by analysing both the activity of uveitis in relation to age and in relation to disease duration to see which was more significant. Although there were some similarities in the uveitis activity patterns in both approaches, we found a significant change in mean cell grade over age, which was not observed in relation to the duration of uveitis. We did find a peak of uveitis activity 9 years after the diagnosis, at which time 74% of the patients were in their estimated puberty years. This might also explain the increase in topical corticosteroid use with longer disease duration. Although our observations suggest an influence of puberty, the exact cause of this second phase of uveitis activity is not yet clarified. One can speculate that hormonal changes during puberty might have an effect on the immune system, resulting in an increase of uveitis activity. It is known that testosterone can have an anti-inflammatory effect by reducing macrophage proliferation, whereas oestrogens can have a proinflammatory effect by stimulating immunoglobulin (Ig)G and IgM production

in mononuclear cells in patients with systemic lupus erythaematosus.[18] Support for the hypothesis of hormonal involvement is that serum levels of testosterone and its precursor dehydroepiandesteronesulfate were decreased in pubertal JIA patients (age 15-18 years).[19] However, serum 17-β oestradiol was not elevated in JIA patients,[19] in contrast to rheumatoid arthritis and systemic lupus erythaematosus patients.[18] Furthermore, pro-inflammatory cytokines were not elevated in aqueous humour of adolescents (10-19 years), compared with younger children who were treated for uveitis.[20] Since children with chronic diseases often have abnormal puberty,[21] a prospective study that would determine puberty stages in each individual and then compare uveitis and arthritis activity before and during puberty would be necessary to confirm and clarify our observations on increased inflammatory activity of JIA-associated uveitis during puberty.

Our study has all the shortcomings of a retrospective study, including for instance missing data over the years, presence of unknown confounders and the inability to prove causal relationships. Also, this study focuses on reversible, provisional markers for disease activity. Consequently, indirect consequences of uveitis activity that might be very important for the patient, such as surgical procedures and loss of visual acuity are not studied here. There is also a referral centre bias, which we tried to minimise by the long minimal follow-up of 2 years. We suspect that with the mean follow-up of 11.4 years, the referral centre bias will be small. Additionally, to increase in uveitis activity, we observed a significant increase in treatment with systemic immunosuppressive medication during estimated puberty years. An increased disease activity was found despite more aggressive systemic medication in estimated puberty years, which might suggest poorer therapy response in long-term disease.

In addition, topical corticosteroid administration was significantly increased in longer disease duration and not significantly related to age. A possible explanation for this controversy might be that after prolonged treatment, habituation or resistance to the medication might occur, resulting in an increased disease activity in a period that happens to coincide with puberty. Low therapy compliance could represent a (partial) cause of the increased uveitis activity during early teenage years. In response, increasing the dose of prescribed corticosteroid eye drops in reported medication in our data was no guarantee that it was actually used by the patient. Especially in teenagers with chronic disease, adherence is problematic. On the other hand, younger children are harder to examine which might cause underdiagnosis in younger children. Systemic anti-inflammatory medication influences both uveitis and arthritis. This study does not differentiate between changes in medication made by the ophthalmologist

or paediatrician because medication prescribed because of arthritis wil l still influence uveitis activity, and because the retrospective nature of the study sometimes made it impossible to distinguish the reason for the medication.

We were unable to detect significant differences in occurrence of cystoid macular oedema and papillitis between prepuberty and estimated puberty years in this small cohort. We did find more hypotony in boys during estimated puberty years compared with prepuberty years. However, in total there were only seven boys with hypotony, of which two occurred after puberty. Because four of these seven patients still had hypotony at the end of follow-up, irreversibility might be one of the causes for this finding that hypotony in boys increased over higher ages. Also, this finding might be disease duration related, because onset of hypotony in boys ranged from 6 to even 12 years after diagnosis of uveitis.

In summary, the results of this study suggest a biphasic course of JIA-associated uveitis, with the second phase of activity during early teenage years and an increase of therapy usage. The cause of the increased activity of uveitis is not yet clarified, but our observations indicate that ophthalmologists who are treating patients with JIA-associated uveitis should be aware of this possible increased activity of uveitis in early teenage years.

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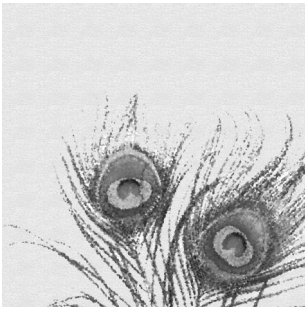

Chapter 6

Relapse rate of uveitis post-methotrexate treatment in juvenile idiopathic arthritis

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Abstract

Purpose: To evaluate the efficacy of methotrexate (MTX) and the effect of its withdrawal on relapse rate of uveitis associated with juvenile idiopathic arthritis (JIA).
Design: Retrospective case series.
Methods: Data of 22 pediatric JIA patients who were being treated with MTX for active uveitis were studied retrospectively. Relapse rate after the withdrawal of MTX was established. Anterior chamber (AC) inflammation, topical steroid use during the first year of MTX treatment, and associations of relapses after the withdrawal were evaluated statistically. Duration of MTX treatment and its withdrawal was determined individually in collaboration with a rheumatologist with an intention to continue the treatment for at least 1 year and to withdraw in case of inactivity of uveitis and arthritis. Inactivity of uveitis was defined as the presence of ≤ 0.5+ cells in the AC.
Results: Eighteen patients (18/22; 82%) showed improvement of their uveitis with a significant decrease in activity of AC inflammation after a minimal period of 3 months of MTX treatment. A topical steroid–sparing effect was observed when MTX was administered for a period of 3 to 9 months. MTX was discontinued because of inactive uveitis in 13 patients. In 9 patients (8/13; 69%) a relapse of uveitis was observed after a mean time of 7.5 months (±SD 7.3). Six patients (6/13; 46%) had a relapse within the first year after the withdrawal. Relapse-free survival after withdrawal of MTX was significantly longer in patients who had been treated with MTX for more than 3 years (P = 0.009), children who were older than 8 years at the moment of withdrawal (P = 0.003), and patients who had an inactivity of uveitis of longer than 2 years before withdrawal of MTX (P = 0.033). Longer inactivity under MTX therapy was independently protective for relapses after the withdrawal (hazard ratio = 0.07; 95% confidence interval 0.01-0.86; P = 0.038), which means that 1-year increase of duration of inactive uveitis before the withdrawal of MTX results in a decrease of hazard for new relapse of 93%.
Conclusions: A high number of patients with inactive uveitis relapse quickly after the withdrawal of MTX. Our results suggest that a longer period of inactivity prior to withdrawal and a longer treatment period with MTX reduce the chance of relapse after withdrawal.

Introduction

Chronic anterior uveitis is known as a severe extra-articular manifestation of juvenile idiopathic arthritis (JIA) and it may occur in up to one third of patients with JIA.[1;2] Chronic anterior uveitis can have a complicated course and it can be potentially deleterious to vision.[3–5]

The aim of treatment of children with JIA-associated uveitis should be control of the inflammation and prevention of the development of sight-threatening complications with an acceptable level of side effects. Topical corticosteroids are the first step of treatment, but local therapy is frequently insufficient and chronic use of topical corticosteroid can enhance the development of cataract and glaucoma. Therefore, systemic immunosuppressive medication is warranted in many cases. Methotrexate (MTX) is an effective treatment option for arthritis in patients with JIA.[6] Several retrospective studies have shown the effectiveness and safety of MTX in the management of uveitis[7–11] in the past decennia, and MTX has been recognized as an effective agent in management of JIA-associated uveitis.[12–15] Although treatment with MTX is generally used for JIA uveitis, there are still some unanswered questions about the optimal duration of MTX therapy and about the risk of relapse of uveitis after discontinuation of MTX therapy. In this study we attempt to clarify these issues.

Patients and Methods

Medical data of MTX-treated patients with JIA-associated uveitis attending a tertiary center for (pediatric) uveitis between 1989 and 2009 were studied retrospectively. JIA was diagnosed and classified by a pediatric rheumatologist in agreement with the International League of Associations for Rheumatology criteria.[16] Diagnosis of uveitis was made by an ophthalmologist specialized in pediatric uveitis. The diagnostic criteria for uveitis were those defined by the International Uveitis Study Group.[17] The JIA-associated uveitis had to manifest before the age of 16 years, and we included all the patients who were treated with MTX for active JIA-associated uveitis (eventually in combination with active arthritis) before the age of 20. The inclusion cut point of 20 years was chosen to prevent losing data of patients who had been treated with MTX late in adolescence. The patients in whom arthritis (and not uveitis) was an indication to start the MTX treatment were not included. All patients in whom MTX was

withdrawn had a minimum follow-up of 1 year after the withdrawal; no patients were excluded because of shorter follow-up.

MTX was prescribed with collaboration of an ophthalmologist and a pediatric rheumatologist. MTX was administered orally or subcutaneously at a dosage of 10 to 15 mg/m2/week. Duration of MTX treatment was determined individually in collaboration with a rheumatologist with an intention to treat the patients for at least 1 year and to withdraw MTX in case of inactivity of uveitis and arthritis. Since there are no standard guidelines for MTX withdrawal available, the drug was usually withdrawn after a variable period of inactivity of uveitis without dose reduction.

The following data were recorded for each patient: age, gender, date of onset of uveitis and arthritis, laterality, subtype of JIA, antinuclear antibody (ANA) serologic status, date and indication of MTX start, date and reason for MTX withdrawal, side effects, treatment with other local or systemic anti-inflammatory therapy, and relapses after the withdrawal. Further, grade of anterior chamber cells and presence of complications were noted. The database was built on patient level with notification of the highest grade of inflammation and dosage of topical steroids in the affected eye. Complications were scored if they were present in any of the affected fellow eyes. Activity of anterior chamber (AC) inflammation (cells) on standard slit-lamp examination was evaluated according to the recommendations of the SUN working group.[18] Additionally, topical corticosteroid requirement was quantified by the number of drops used per day. Activity of AC inflammation and corticosteroid requirement was scored at fixed time points: moment of starting MTX therapy and at 3, 6, 9, and 12 months of MTX use. Activity of uveitis was defined as the presence of ≥ 1+ cells in the AC. Improvement on MTX was considered as at least a 2-step decrease of the cell grade in the AC. Inactivity of uveitis was defined as the presence of ≤ 0.5+ cells in the AC. Relapse of uveitis after withdrawal of MTX was scored in case of presence of 1+ or more cells in the AC in patients with inactive uveitis at the moment of the withdrawal. There was no difference in follow-up schedules pre- and post-MTX withdrawal (approximately every 6 weeks).

Statistical analysis was performed using SPSS version 15.0.1 (SPSS Inc, Chicago, Illinois, USA). Fisher exact test was used for univariate analysis of categorical variables. McNemar test was used to analyze linked dichotomous variables. Wilcoxon test for paired samples was used to analyze means of abnormally distributed linked samples; for independent samples with abnormal distribution Mann-Whitney U test was applied. For correlations between normally distributed continuous variables, Pearson correlation coefficient (r) was computed. Kaplan-Meier survival analysis with a log

rank test was used to analyze survival curves and to compare groups. Cox proportional hazard regression was applied for multivariate analysis of variables significant in univariate analysis and adjusted for gender as a potential confounder. P values of less than 0.05 were considered statistically significant. All significances are 2-tailed. In the presentation of the results we used mean if the data were normally distributed and median if they were not.

Results

Twenty-two patients with active uveitis at initiation of MTX were included in the study. General baseline characteristics of the study population are listed in Table 1.

TABLE 1. General Baseline Characteristics of Patients With Uveitis Associated With Juvenile Idiopathic Arthritis Treated With Methotrexate

Characteristics	Number of Patients, n (%)	
	Initiation of MTX N = 22	Withdrawal of MTX N = 13
Active uveitis	22 (100)	NA[a]
Gender		
Female n (%)	15 (68)	9 (69)
Age in years		
At onset of arthritis, median (range)[b]	3.3 (1.5-11.3)	3.2 (1.7-11.3)
At onset of uveitis, median (range)	4.0 (1.9-9.5)	4.1 (1.9-7.4)
At start MTX (mean ± SD)[c]	8.0 ± 4.0	6.0 ± 3.0
Duration of uveitis in years		
At start MTX, median (range)	1.8 (0.1-10.9)	0.6 (0.1-7.0)
Type of JIA		
Oligoarticular n (%)	20 (91)	12 (92)
Polyarticular n (%)	1 (4)	1 (8)
Psoriatic n (%)	1 (4)	NA
ANA status		
Positive n (%)	20 (91)	11 (85)
Laterality		
Bilateral n (%)	18 (81)	12 (92)

ANA = antinuclear antibody; JIA = juvenile idiopathic arthritis; MTX = methotrexate; NA = not applicable; SD = standard deviation.

[a]All patients had inactive uveitis at the moment of withdrawal of MTX.

[b]Abnormal distribution.

[c]Normal distribution.

Clinical characteristics at initiation of MTX therapy

Duration of uveitis before starting MTX therapy ranged from 0.1 to 10.9 years (median 1.8 years) (Table 1). In 50% of patients (11/22) MTX was initiated because of active uveitis and in another 50% because of a combination of active uveitis and arthritis. In all but 1 patient MTX was initially administered orally. Activity of the AC inflammation at the start of MTX therapy ranged from 1+ to 3+ cells (Table 2). Ocular complications present at start of MTX included posterior synechiae (15/22; 68%), band keratopathy (5/22; 23%), cystoid macular edema (4/22; 19%), papillitis (4/22; 19%), and glaucoma (2/22; 9%). Five patients (23%) underwent cataract surgery before the start of MTX.

Uveitis activity during MTX therapy

Eighteen patients (18/22; 82%) showed improvement of their uveitis activity with at least a 2-step decrease of the AC cells within the first year (Table 2). Of those; 15 patients achieved inactivity of uveitis within the first year of MTX therapy (median = 0.5 year), while 3 patients improved on MTX without reaching complete control of inflammation. A significant decrease in activity of uveitis in the whole group was seen at 3 months of MTX use (Table 2). Patients who improved on MTX treatment did not differ significantly from patients without improvement in age, gender, duration of uveitis, arthritis, ANA serologic status, MTX dosage, or other therapeutic strategies.

Systemic and topical medication during MTX treatment

At the moment of initiation of MTX, 4 patients (4/22; 18%) were treated with systemic corticosteroids. In 3 of them (3/4; 75%) steroid treatment was withdrawn within 2 months of commencement of MTX and the other patient required continuation of prednisone during MTX treatment for achieving inactivity of uveitis.

At the initiation of MTX therapy, 20 patients (20/22; 91%) were treated with topical steroids (prednisolone 1%) with a median of 2 drops/day (range of 1-6 drops/day). The local corticosteroid therapy could be decreased in 13 of 22 patients (59%) with a significant topical steroid-sparing effect from 3 up to 9 months of start of MTX therapy. The statistical significance of steroid-sparing effect was lost at 12 months of MTX therapy (Table 2).

None of the patients were using other immunosuppressive agents during the first year of MTX therapy. An anti–tumor necrosis factor (TNF)- α agent was added in 2 patients after 8 and 9 years of MTX therapy. Cyclosporine was added to MTX in 1 patient after 2 years of MTX therapy. Nine patients received periocular steroid injections (however, only 2 within the first year of MTX therapy).

TABLE 2. Anterior Chamber Inflammation Activity Grade and Therapy With Topical Steroids During Methotrexate Therapy in Children With Uveitis Associated With Juvenile Idiopathic Arthritis

	MTX At Start	MTX 3 Months	MTX 6 Months	MTX 9 Months	MTX 12 Months
Anterior chamber activity					
Patients with active anterior uveitis, n (%)	22 (100)	10 (45)	8 (36)	8 (36)	7 (32)
P value[a]	NA	<.001[b]	<.001[b]	<.001[b]	<.001[b]
Median of anterior chamber cell activity grade (range)[c]	1 (1-3)	0.5 (0-2)	0.5 (0-3)	0.5 (0-2)	0.5 (0-2)
P value[d]	NA	.001[b]	.037[b]	.003[b]	<.001[b]
Topical steroids					
Patients on topical steroids,[e] n (%)	20 (91)	21 (95)	19 (86)	18 (82)	18(82)
Dosage of topical steroids per day; median drops (range)	3 (0-6)	2 (0-4)	2 (0-6)	2 (0-6)	3 (0-4)
P value[d]	NA	.011[b]	.017[b]	.039[b]	.146[b]

MTX = methotrexate.
[a]*P* values computed using McNemar test.
[b]Compared with the moment of start MTX.
[c]Anterior chamber activity is scored according to the recommendations of SUN working group.[18]
[d]*P* values computed with Wilcoxon test.
[e]Prednisolone 1% was administered in all patients on topical steroids.

Results after withdrawal of MTX

General baseline characteristics of patients in whom MTX was withdrawn are presented in Table 1. MTX was discontinued in 13 patients with inactive uveitis after 1.5 years mean duration of inactivity of uveitis (range 1 month to 3 years) and 3.1 years mean duration of the therapy. Follow-up after the withdrawal ranged from 1.0 to 11.1 years with a median of 1.7 years. Median age at withdrawal of MTX was 8.6 years (range 3.5-16.5 years).

In 9 of these patients (9/13; 69%) a new relapse of uveitis was observed after a mean time of 7.5 months (±SD 7.3), with the first relapse after 1 month of withdrawal of MTX. In all cases a new relapse took place within 2 years after the discontinuation of MTX, while 6 patients (6/13; 46%) relapsed within the first year after the withdrawal.

Relapse-free survival after the withdrawal of MTX was significantly longer in patients who had been treated with MTX longer than 3 years (P = 0.009; Figure 1), children who were older than 8 years at the moment of the withdrawal (P = 0.003; Figure 2), and patients who had an inactivity of longer than 2 years before the withdrawal of MTX (P = 0.033; Figure 3).

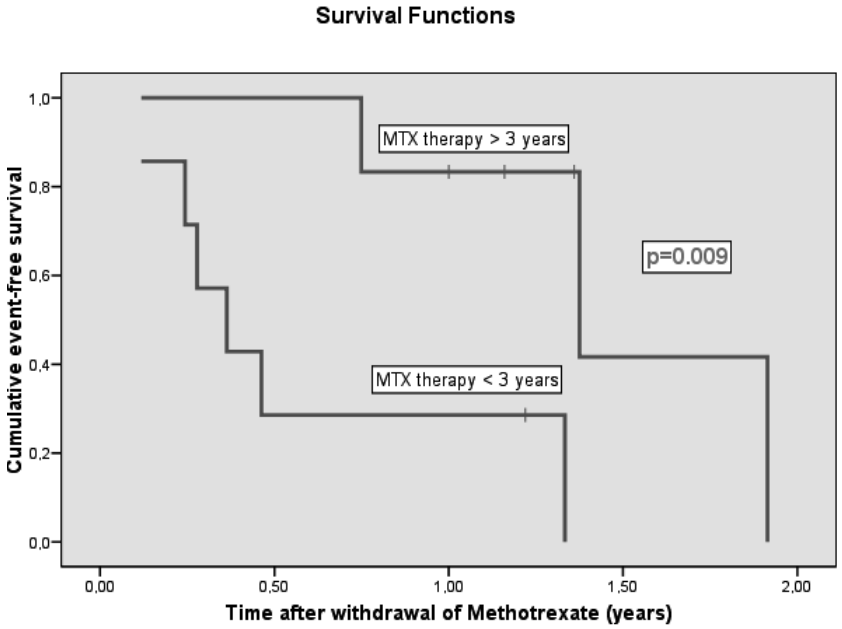

Figure 1. Survival plot for relapses of uveitis after withdrawal of methotrexate (MTX) in patients with inactive uveitis according to the total duration of MTX therapy.

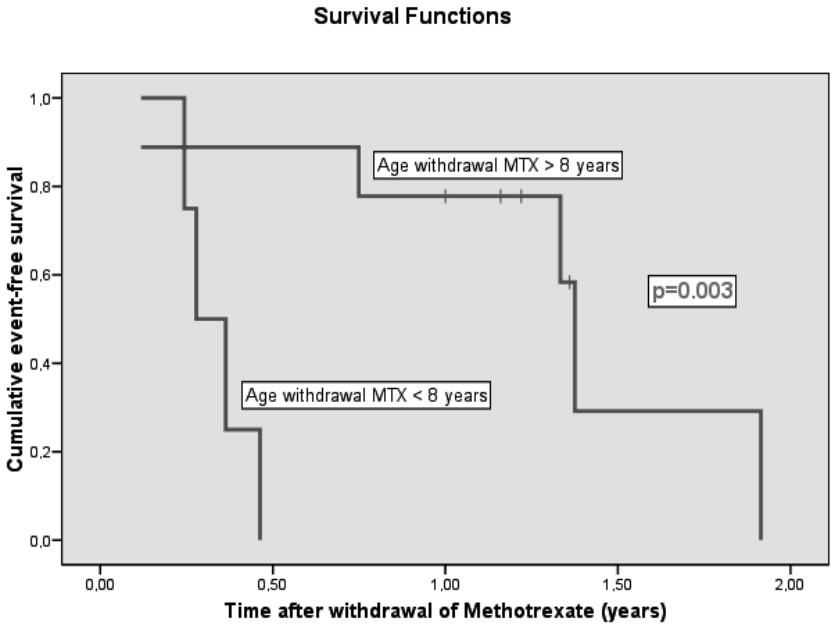

Figure 2. Survival plot for relapses of uveitis after withdrawal of methotrexate (MTX) in patients with inactive uveitis according to the age at withdrawal.

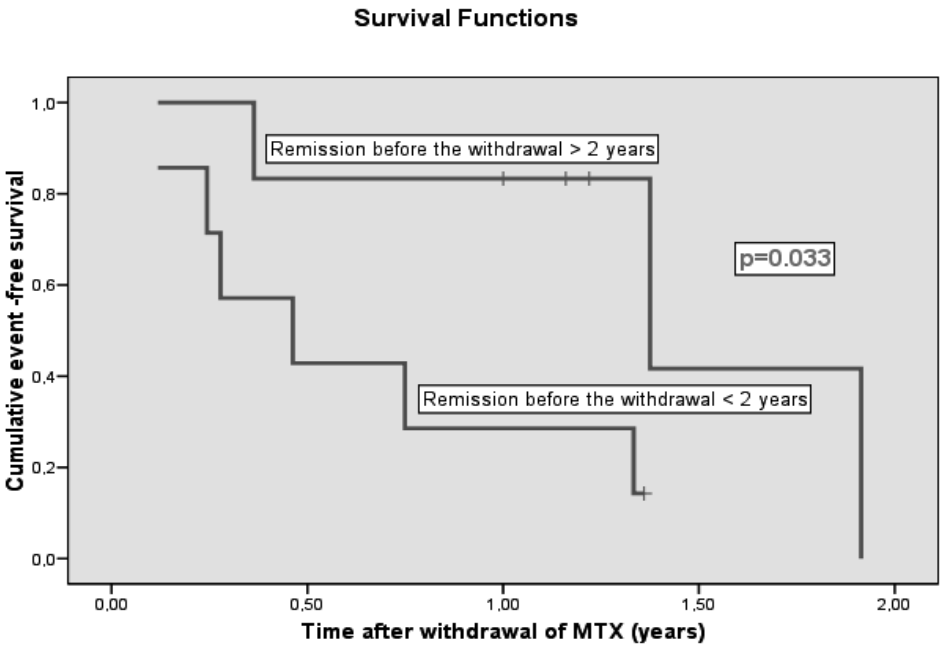

Figure 3. Survival plot for relapses of uveitis after withdrawal of methotrexate (MTX) in patients with inactive uveitis according to the duration of inactivity before withdrawal.

Furthermore, in our series, time interval to relapse after the withdrawal correlated linearly with duration of inactive uveitis before the withdrawal (r = 0.78; P = 0.014), age at the withdrawal (r = 0.81; P = 0.009), and duration of MTX therapy (r = 0.73; P = .027). A multivariate analysis of these factors together (additionally adjusted for gender) pointed out duration of inactive uveitis before the withdrawal as an independent factor associated with a chance of relapse after the withdrawal of MTX (Table 3). A longer period of inactivity during the MTX therapy was associated with less chance of relapse after MTX withdrawal (hazard ratio = 0.07; 95% confidence interval 0.01-0.86; P = 0.038) (Table 3). In other words, 1-year increase of duration of inactive uveitis before the withdrawal of MTX results in a decrease of hazard for new relapse of 93%. All but 1 patient used topical steroids after the withdrawal of MTX. No one was treated with other immunosuppressive agents before, simultaneous with, or after the withdrawal of MTX. In 8 patients (8/13; 62%) MTX was restarted after a mean time interval of 1.4 years (±SD 0.8) after the initial withdrawal, in 2 of them in combination with an anti-TNF- α agent.

Side effects

Eleven of 22 patients (50%) experienced side effects during MTX treatment. These in-

TABLE 3. Output of Cox Proportional Hazard Regression for Multivariate Analysis of Risk Factors for Relapse of Uveitis After Withdrawal of Methotrexate in Juvenile Idiopathic Arthritis

Variable	B	P Value	Exp(B)	95% CI for Exp(B)	
				Lower	Upper
Age at MTX withdrawal	−0.19	0.456	0.83	0.5	1.37
Duration of MTX therapy	−0.81	0.124	0.45	0.16	1.25
Duration of inactivity of uveitis before MTX withdrawal	−2.70	0.038	0.07	0.01	0.86
Gender	−1.19	0.464	0.31	0.01	7.33

B = unstandardized regression coefficient; CI = confidence interval; Exp(B) = hazard ratio; MTX = methotrexate; SE = standard error.

cluded gastrointestinal (nausea, stomach pain, etc) complaints (n = 7; 32%), elevated liver enzymes (n = 2; 9%), or a combination of both (n = 2; 9%). In 3 patients the gastrointestinal side effects improved when administration of MTX was switched to subcutaneous injections. Elevation of liver enzymes was transient in 3 patients. In the majority of cases the reported side effects were relatively mild and transient, and led in only 2 cases to the withdrawal of MTX (9%; gastrointestinal side effects [n = 1] an reversible elevated liver enzymes [n = 1]).

Discussion

Our study demonstrates that the majority (69%) of patients with inactive uveitis at the moment of withdrawal of MTX develop a relapse at a later stage. Most of them had a relapse of uveitis within the first year after discontinuation of MTX therapy. The data also indicate that a longer period of inactivity of uveitis before withdrawal, together with older age and/or longer treatment with MTX, might protect against relapses of uveitis. Furthermore, our study confirms the earlier results about the efficacy of MTX in the reduction of inflammation in uveitis associated with JIA and its topical steroid–sparing effect.[12–15]

Optimal duration of MTX treatment and timing of safe MTX withdrawal in patients with uveitis associated with JIA are not known, nor is the risk of relapse of uveitis after the withdrawal of MTX. Clinical criteria for safe withdrawal of MTX are not identified, but it has been recommended to prolong the systemic treatment with MTX for "a considerably long time."[14] However, exact guidelines are, unfortunately, lacking. Previously Foeldvari and Wierk reported on 6 patients in whom monotherapy of MTX was discontinued after 12 months of complete inactivity of uveitis. In their series, 2 patients (33%) had relapses: after 3 and 8 months after the withdrawal.[13] It is remarkable that the relapse rate in our study was 2-fold higher, which might be explained by the fact that not everyone in our series had completed 12 months of inactivity before the withdrawal of MTX. However, duration of post-withdrawal follow-up in the study of Foeldvari is unclear, and relapses after 7.5 months post-withdrawal (the last follow-up point reported) are possible.

In our series we have observed a correlation of the duration of inactivity before the withdrawal of MTX and time to relapse after the withdrawal. Our results indicate that prolonged duration of inactivity before withdrawal may protect against early relapse. To be precise: 1-year increase of duration of inactive uveitis before the withdrawal of MTX resulted in a decrease of hazard for new relapse of 93%. We also observed a relation between early relapses and shorter duration of MTX therapy and younger age at the withdrawal. Despite the small size of the series, multivariate analysis suggested that, out of the mentioned factors, duration of inactivity before the withdrawal was an independent risk factor for relapse of uveitis. However, this observation needs to be evaluated in the future in a larger prospective study.

MTX is effective in improving activity of AC inflammation in patients with JIA-associated uveitis, as shown in our series and previous studies.[12–15] Significant decrease in topical steroid requirement is important since their use is associated with development of such ocular complications as secondary glaucoma and cataract. As shown before, children on MTX therapy develop cataract later in the course of their disease;[19] however, another report shows that new complications might occur during treatment with MTX.[14]

It is noteworthy that the percentage of patients with side effects of MTX varies between our study (50%) and other studies (9% and 43%).[14;15] One explanation for more gastrointestinal side effects in our study could be that most of our patients had taken MTX orally, in contrast to other studies, where MTX was administered subcutaneously.[15] Important limitations of the current study should be addressed. First of all, the retrospective design of this study implicates that the findings should be taken with

caution because of the methodological limitations. Not all patients had monotherapy with MTX, so evaluation of isolated effect of MTX is complicated. However, additional systemic steroids could be decreased in most patients shortly after the initiation of MTX, and only 1 patient needed continuation of this therapy during the first year on MTX. Furthermore, no other immunosuppressive agents were introduced in the first year of MTX treatment. Obviously, the exact cell count and flare are impossible to note in a retrospective study. Therefore, we have chosen to classify relapses in cases with at least 1+ cell in the aqueous. In consequence, some patients with a low-grade uveitis (0.5+ cell in AC) would not be scored as a relapse. Relapse percentage after the withdrawal of MTX could also be underestimated because relapses after the end of follow-up cannot be excluded. However, all patients completed a 1-year follow-up after withdrawal and the relapse rate during the first year after withdrawal of MTX could be calculated. Small study size limits the analysis, increases risk of type II error, and makes it impossible to account for all potential confounders. However, in the circumstances where prospective studies are lacking, the current study provides important clinical information and helps to improve treatment guidelines. Combining our results together with the results of others,[13;14] we would recommend considering prolonged administration of MTX with a goal to reach a long-term period of inactivity of uveitis before withdrawal. Our results indicate that the period of inactivity before withdrawal should be preferably longer than 2 years.

In conclusion, our study demonstrates that after withdrawal of MTX, the relapse of uveitis occurs in the majority of JIA patients within the first year. Our results indicate that prolonged treatment with MTX and a longer period of inactivity before the withdrawal may be desirable to minimize new relapses after the withdrawal. Large prospective studies are necessary to evaluate our findings and to determine safe criteria for MTX withdrawal in children with JIA.

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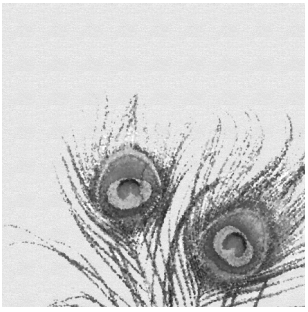

Chapter 7

Intraocular biomarker identification in uveitis associated with juvenile idiopathic arthritis

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Abstract

Purpose: To investigate the presence of disease specific protein profiles in aqueous humor (AH) from patients with uveitis associated with juvenile idiopathic arthritis (JIA).
Methods: Aqueous humor (AH) (n=73) and serum (n=105) samples from a total of 116 children were included. The samples were analyzed using Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-ToF MS). Prior to statistical analysis samples were divided into the following four groups: definitive JIA; suspected JIA (uveitis typical for JIA but not meeting International League against Rheumatism (ILAR) criteria); other uveitis entities and non-inflammatory controls. Biomarker identification was performed using the SELDI-ToF Biomarker Analysis Cluster Wizard (Ciphergen Express Software 3.0) followed by Expression Difference Mapping to statistically analyse the relevance of potential biomarkers. Biochemical identification of biomarkers was performed by polyacrylamide gel protein separation, followed by liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis of trypsin-digested peptides.
Results: Twenty-six and 21 protein peak clusters could be detected in AH and serum, respectively. In the definitive JIA group three protein peaks at mass/charge (m/z) 6,672, 8,725 and 13,762 were detected in AH which were expressed significantly more often and at higher intensities compared to the other uveitis entities group and the non-inflammatory controls. Definitive JIA and suspected JIA samples showed similar protein profiles in AH and did not differ in any of the peak cluster intensities. For the protein at m/z 13,762 no correlating peak in serum could be identified. By the intraocular presence of the protein peak at m/z 13,762 and absence of the peak at m/z 8,255 definitive JIA samples could be distinguished from other uveitis entities and controls with a sensitivity of 64% and a specificity of 96%. Using this algorithm, 5 out of 8 (63%) patients with suspected JIA could be classified together with definitive JIA patients. Mass spectrometric analysis revealed the peak at m/z 13,762 as transthyretin (TTR). TTR was significantly over-expressed in both patients with definitive and suspected JIA and its quantitative expression was associated with activity of the uveitis.
Conclusions: The AH of patients with JIA-associated uveitis has a distinct expression profile compared with other uveitis entities and non-inflammatory controls. This expression pattern was similar for patients who were on clinical grounds suspected for JIA-association of their uveitis but did not meet the ILAR criteria completely. TTR was identified as a biomarker for JIA, and its involvement in the pathogenesis of JIA-uveitis needs further investigation.

Introduction

Juvenile idiopathic arthritis (JIA) is the main underlying cause of uveitis in children.[1] Uveitis in JIA is characterized by mostly anterior location, bilateral involvement, an insidious onset and a subclinical course. Most children with JIA-uveitis are being diagnosed after the diagnosis of arthritis has been made. However, in 23% of children, uveitis can be the first sign of JIA with arthritis being diagnosed months to years after the diagnosis of uveitis.[2;3]

Keeping in mind this atypical presentation of JIA and the absence of a definitive diagnostic test, there is a strong rationale for efforts to identify specific biomarkers in the ocular fluid of children with JIA-associated uveitis. Proteomic techniques based on mass spectrometry approaches are nowadays being widely applied in autoimmune diseases for the identification of biomarkers which could serve for diagnostic, prognostic or even therapeutic purposes.[4] Although biomarker profiling of aqueous humor (AH) has not frequently been performed, the applicability of Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-ToF MS) for this purpose has been demonstrated.[5-8]

In this report we provide data on proteomic analysis of paired AH and serum samples of pediatric uveitis patients, including definitive JIA, JIA-uveitis suspected on clinical grounds, other uveitic entities, and non-inflammatory pediatric controls with cataract and/or glaucoma.

Patients and Methods

Study participants

A total of 116 children who visited the Department of Ophthalmology of the University Medical Center Utrecht (UMCU), the Netherlands, between 2004 and 2010, participated in this study. The diagnosis of uveitis was made by an ophthalmologist specialized in childhood uveitis. Uveitis was evaluated according to the criteria of the International Uveitis Study Group.[9] Uveitis was diagnosed if 1+ or more cells in the anterior chamber were seen over at least two eye examinations. All children from the uveitis group were additionally evaluated by a pediatric rheumatologist and/or immunologist to confirm or to exclude a systemic disorder. The diagnosis of JIA was based on the criteria of the International League against Rheumatism (ILAR).[10]

For analysis, both AH and serum samples were divided into four groups: definitive JIA-associated uveitis, suspected JIA-associated uveitis, other uveitis entities (including intermediate, infectious and undefined uveitis) and non-inflammatory controls (congenital cataract, congenital glaucoma and traumatic cataract). Table 1 shows the available ocular fluid and serum samples within these specific groups. Table 2 shows the distribution of age, gender and duration of uveitis within the groups of available AH samples.

Definitive JIA-associated uveitis was defined as uveitis in a child who was diagnosed with JIA (according to the ILAR criteria) by a pediatric rheumatologist. Suspected JIA-uveitis was defined as silent, bilateral insidious anterior uveitis when other infectious and/or systemic causes were excluded by examination by a pediatric rheumatologist and/or immunologist (10/10 patients), serological tests (10/10 patients) and AH analysis (3/10 patients, all seronegative for antinuclear autoantibodies (ANA); in 2 of these patients the samples were obtained during an elective intraocular surgery). In these patients a diagnosis of JIA could not be made because of incomplete correlation with ILAR criteria, although their clinical features of uveitis were consistent with JIA-associated uveitis. Moreover, ANA was positive in 5 out of 10 children with suspected JIA-associated uveitis. Active uveitis at the time of sampling was defined as at least trace cells in the AH on examination prior to AH puncture in the same eye.

The study was approved by the independent Institutional Review Board (IRB) of the UMCU. The legal guardians of all study subjects from whom samples were obtained during surgery signed a written Informed Consent document.

Sample collection

AH and serum samples were collected at the UMCU, the Netherlands. From 62 patients paired AH and serum samples were collected. From 11 patients only AH was collected, whereas from 43 patients only serum samples were available (Table 1). AH samples from children with uveitis were obtained for diagnostic purposes (n = 25) or during therapeutically necessary intraocular surgery (n = 48). In the JIA and suspected JIA groups samples were obtained during therapeutically required surgery in all but one patient. This patient had suspected JIA-associated uveitis but did not meet ILAR critera. The AH sample from this patient was collected to exclude an infectious etiology of uveitis before initiating immunosuppressive therapy. All samples from control patients without uveitis were collected during elective intraocular surgery. AH samples were extracted by an experienced ocular surgeon using a binocular microscope. The samples were stored at -80°C within five hours of collection.

Table 1. Overview of available samples within the patient groups.

Number of patients / Material available	Total N = 116	JIA N = 17	Suspect JIA N=10	Other uveitis N=62	Controls N=28
AH (N (%))	73 (63)	14 (82)	8 (80)	31 (50)*	20 (71)†
Serum (N (%))	105 (91)	16 (94)	9 (90)	59 (95)	21 (82)
Paired AH and serum (N (%))	62 (53)	13 (76)	7 (70)	27 (44)	15 (54)

* including 10 intermediate, 3 infectious and 18 uveitis of undefined etiology
† including 11 congenital cataract, 3 traumatic cataract and 6 congenital glaucoma

Table 2. General characteristics of the patient groups based on aqueous humor (AH) availability.

Number of patients / Characteristics	Total N = 73	JIA N=14	Suspect JIA N=8	Other uveitis N=31	Controls N=20	p-value *	p-value JIA vs. JIA suspect †	p-value JIA vs. other uveitis †	p-value JIA vs. controls †
Females (N (%))	40 (55)	10 (71)	6 (75)	16 (52)	8 (40)	0.199	-	-	-
Age in years (median; range) ‡	10.6 (0.1 - 23.9)	11.5 (4.9 - 23.9)	11.1 (8.1 - 16.6)	13.8 (0.1 - 17.8)	4.2 (0.4 - 15.9)	<0.001	1.000	0.831	0.003
Duration of uveitis in years (median; range) ‡	1.5 (0 - 22.3)	8.1 (0.9 - 22.3)	3.5 (0.5 - 11.3)	0.5 (0 - 7.7)	NA	<0.001	0.630	0.003	NA

* Pearson Chi-square test was used for analysis of categorical variables. Mann-Whitney test and Kruskal-Wallis test were used to compare medians between the groups.
† Bonferroni correction for multiple testing is applied.
‡ at the time of aqueous puncture. NA: not applicable

SELDI-ToF MS analysis

Normal Phase (NP20) Protein chip arrays (Bio-Rad, Hercules, USA) were used and pre-treated according to standard manufacturer's procedures. Each array was conditioned with 1 µl of deionized water. Then 1 µl of AH or serum was applied onto the array and allowed to mix with the water. After air drying the arrays were washed twice with 5 µl of deionized water to remove salts. When the spots were dry 1 µl of sinapinic acid (SPA), an energy absorbing molecule (EAM), was applied twice on every spot with an interval of 5 to 10 minutes. Each array was analyzed with a standard protocol where 350 shots were fired on each spot using a laser intensity of 6000 nJ, a deflector setting at m/z 3,000, a detector sensitivity of nine and a molecular mass detection range from m/z 1,000 to 200,000 . The optimization range was from m/z 3,000 to 50,000. The target mass was set at a m/z of 10,000 Th. Calibration was performed with the All-in-One Protein Standard calibrant (Cyphergen Biosystems, Inc, Fremont, CA, USA). The data were clustered in JIA, JIA-suspect, other uveitis and non-inflammatory controls.
Automatic peak detection by Cluster Wizard (Ciphergen Express software 3.0) was performed using the settings of four times signal-to-noise ratio and a valley depth of three. The cluster width was specified as dynamical adjustment of the mass window based on the width of the peak. In order to be assigned as a peak, it had to be present in 5% of total AH samples and 10% of total serum samples. For automatic peak detection within JIA and suspected JIA samples only, the peak had to be present in at least 20% of the spectra. To ensure accurate statistical analysis, spectra were manually inspected for mislabeling of peaks and adjusted if necessary.

Data analysis

ProteinChip Expression Difference Mapping (Ciphergen Biosystems, Freemont, USA) was used for the initial selection of candidate biomarkers. Unsupervised multivariate hierarchical cluster analysis was used for assessment of sample relationships. The proximity of spectra and the branching patterns in the dendrogram reflect sample similarity. The cluster lists were exported to SPSS version 15.0.1 (SPSS Inc, Chicago, Illinois, USA) for further statistical analysis. The Kruskal-Wallis test and Mann-Whitney U-test were used for non-parametric analysis of differences between the groups. For comparison of more than two groups Bonferroni correction of p-values was applied, that is calculated p-values were multiplied by the number of comparisons. Two-way ANOVA and binary logistic regression were used for multivariate analysis and interaction effects. Spearman's rho correlation coefficient was computed to estimate

correlations between peak intensities. The Pearson Chi-square test or the Fisher's exact test was used to compare associations between categorical variables when appropriate. P-values of < 0.05 were considered significant.

Protein purification and identification

Fourteen samples of patients with JIA and suspected JIA containing the marker at m/z 13,762 (Figure 1) were pooled yielding a volume of almost 2 ml of undiluted AH. Confirmation of the presence of the peak at m/z 13,762 was performed using the same array preparation protocol as described above. The arrays were read on a Proteinchip Enterprise 4000 system with target m/z at 13,000, matrix attenuation at m/z 2,500 and m/z range from 0 - 40,000. The protein concentration of the pooled sample was determined by both the bicinchoninic acid (BCA) protein assay and the Bradford protein assay to calculate average protein concentration. Protein concentration in the pooled sample was estimated to be 1375 mg/ml. Protein purification was performed by denaturing SDS-Page (12% BisTris pre-cast gels, Invitrogen). The gels were fixed and stained with coomassie brilliant blue (Sigma) for 30 minutes. Protein bands resolved in the molecular weight range of interest were excised from the polyacrylamide gel. Proteins were digested with trypsin as previously described.11 Prior to LC/MS/MS analysis samples were resuspended in 30 ml of 5% acetonitrile (ACN)/ 0.1% formic acid. Peptides were resolved by reverse phase chromatography (Biobasic column, ThermoScientific USA; 180uM x 15mm) over a 30 minutes ACN gradient at a flow rate of 2 ml/min using the Surveyor LC system (ThermoScientific) followed by MS/MS spectra acquisition using an LCQ Deca XP Plus (ThermoScientific).

Raw data files were converted into Mascot generic files using the MassMatrix File Conversion Tool (Version 2.0) for input into the Mascot searching algorithm (Matrix Science). The data files were searched against SwissProt (v. 2012_07) with human taxonomy using the following search criteria: tryptic peptides with up to one missed cleavage and carbamidomethylation of cysteines and oxidation of methionines, which were set as variable modifications.In parallel to protein digestion equivalent gel bands were excised for passive elution and determination of actual protein mass. Excised bands were cut into 1 mm^3 sized pieces and washed with 100 mM ammonium bicarbonate. The washing solution was discarded and gel slices dehydrated with 50% ACN/50% 100mM ammonium bicarbonate. Solvent was discarded and gel pieces air dried at 420C to remove all traces of solvent. A minimal volume of protein elution solution (50% formic acid, 25% ACN, 15% isopropanol, 10% water) was added to cover

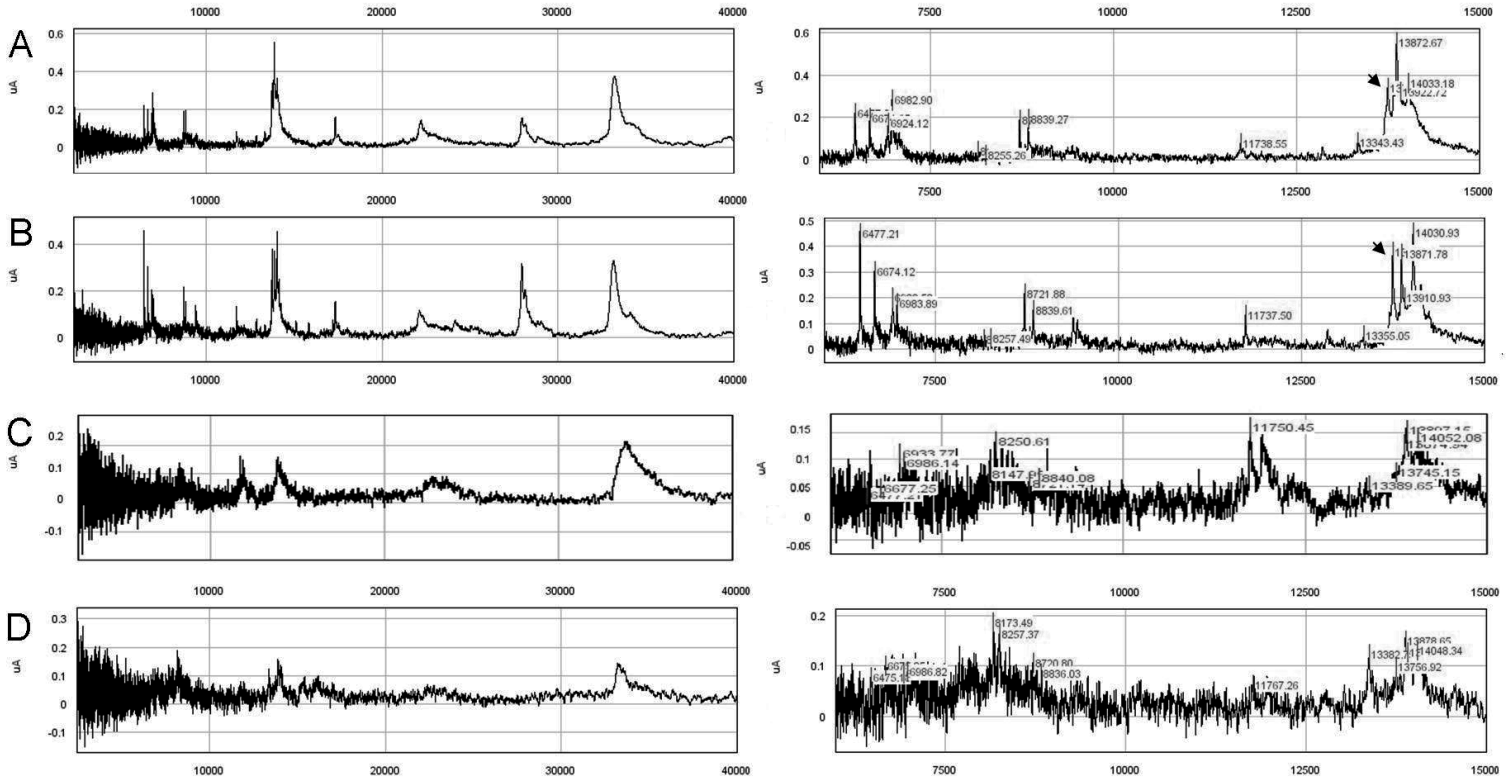

Figure 1. Aqueous humor SELDI-ToF MS spectra representative for the 4 study groups: A - Juvenile Idiopathic Arthritis (JIA) associated uveitis; B - uveitis clinically suspect for JIA; C - other pediatric uveitis; D - non-inflammatory pediatric controls. On the left hand side an overview of the peaks in the range 2500-40000 Da are displayed. On the right hand side the same spectra, now including peak labels, are shown zoomed-in from 6000 to 15000 Da. The peak at m/z 13,762 is labeled with an arrow in A and B.

the gel pieces. Proteins were extracted for 1 hour with 30 minutes of sonication. The supernatant was collected and analyzed by SELDI-ToF MS directly on a gold array without pre-conditioning.

Results

SELDI-ToF MS cluster peak analysis

To investigate the presence of possible biomarkers for JIA-associated uveitis and to help the identification of candidates for specific intraocular biomarkers in JIA-associated uveitis cluster peak analysis of the SELDI-ToF MS data was performed. Figure 1 displays a representative peak profile for each of the four groups within the m/z range of 2,500 to 40,000 Da, including a close up in the range of 5,000 to 15,000 Da.

When analyzing all AH samples, 26 protein peak clusters were detected. Of these 15 showed significant differences in expression levels between the four study groups (Supplemental Table 1). When analyzing the absence or presence of these 15 protein peaks, eight of them at m/z 6,475; 6,672; 8,725; 8,840; 13,762; 13,914; 22,313 and 27,981 displayed significant differences between the groups (Table 3). In the JIA group both the presence and expression levels of the peaks at m/z 6,672; 8,725 and 13,762 were significantly increased compared to other uveitis entities and controls (Table 3). Definitive JIA and suspected JIA samples showed similar protein profiles and these groups did not differ in any of the peak cluster intensities or detection frequencies (Supplemental Table 1 and Table 3).

Spearman's rho correlation coefficient analysis of the peak intensities at m/z 6,672; 8,725 and 13,762 in JIA-uveitis patients revealed several significant positive and negative correlations which are presented in Table 4. The peak at m/z 13,762 showed both positive and negative significant correlations. Positive correlations were found with peaks at m/z 6,989; 13,884; 13,914 and 27,981 (Spearman's rho correlation coefficient between 0.754 and 0.837). Negative correlations were observed with the peaks at m/z 8,161 and 8,255 and 16,171 (Spearman's rho correlation coefficient between -0.723 and -0.873; Table 4). Two other peaks at m/z 6,672 and 8,725, which were significantly over-expressed in JIA-associated uveitis were not significantly correlated to the peak at m/z 13,762 (Table 4).

Cluster analysis of serum revealed 21 peak clusters (Supplemental Table 1). Significant differences in expression intensity were only detected between JIA-uveitis and the other uveitis entities. The peak at m/z 13,762, which was significantly up-regulated in

Table 3. Overview of the expression and detection by SELDI-ToF MS of the protein peaks in aqueous humor (AH) and serum with significant differences between the study groups: uveitis associated with juvenile idiopathic arthritis (JIA); uveitis suspect for association with JIA, other pediatric uveitis entities and pediatric non-inflammatory controls).

Protein peaks m/z	Average peak intensity in AH N=73								Detected peaks in AH N=73							
	JIA N=14	Suspect JIA N=8	Other uveitis N=31	Control N=20	p-value*	JIA versus suspect JIA p-value†	JIA versus other uveitis p-value†	JIA versus control p-value†	JIA N=14 (%)	Suspect JIA N=8 (%)	Other uveitis N=31 (%)	Control N=20 (%)	p-value*	JIA versus suspect JIA p-value†	JIA versus other uveitis p-value†	JIA versus control p-value†
6,475	0.103	0.234	0.066	0.064	**0.004**	0.738	**0.048**	0.081	9 (64)	4 (50)	4 (13)	1 (5)	**< 0.001**	1.000	**0.003**	**<0.003**
6,672	0.106	0.211	0.05	0.064	**<0.001**	1.000	**0.003**	**0.03**	9 (64)	5 (62)	2 (7)	1 (5)	**< 0.001**	1.000	**<0.003**	**<0.003**
8,725	0.096	0.107	0.063	0.061	**0.011**	1.000	**0.024**	**0.012**	7 (50)	3 (38)	3 (10)	ND	**< 0.001**	1.000	**0.015**	**0.003**
8,840	0.096	0.096	0.068	0.068	**0.035**	1.000	**0.045**	0.117	10 (71)	3 (38)	5 (16)	ND	**< 0.001**	0.561	**0.003**	**<0.003**
13,762	0.232	0.229	0.12	0.087	**0.004**	1.000	**0.045**	**0.021**	9 (64)	5 (63)	3 (10)	ND	**< 0.001**	1.000	**<0.003**	**<0.003**
13,914	0.311	0.237	0.199	0.135	**0.008**	1.000	0.351	**0.018**	4 (29)	7 (88)	12 (39)	5 (25)	**0.018**	0.072	1.000	1.000
22,313	0.113	0.108	0.09	0.072	**<0.001**	0.657	**0.03**	**0.003**	13 (93)	8 (100)	25 (81)	12 (60)	0.052	1.000	1.000	0.150
27,981	0.091	0.117	0.051	0.036	**0.018**	1.000	0.285	**0.03**	10 (71)	5 (63)	12 (39)	3 (15)	**0.001**	1.000	0.171	**0.03**

ND: not detected; JIA: juvenile idiopathic arthritis; AH: aqueous humor

P- values for the intensities are calculated using Mann-Whitney U-test and for the detection using Fisher exact test.

* - Mann-Whitney test and Kruskal-Wallis test were used to compare medians between the groups.

† - Bonferroni correction for multiple testing is applied

Supplemental Table 1. Overview of the average peak intensities of detected peak clusters in aqueous humor (AH) and serum samples of patients with uveitis associated with juvenile idiopathic arthritis (JIA), uveitis suspect for association with JIA, other uveitis entities and non-inflammatory controls.

Protein peaks m/z	Average peak intensity in AH (N=73)								Average peak intensity in serum (N=105)							
	JIA N=14	Suspect JIA N=8	Other uveitis N=31	Control N=20	p-value*	JIA versus suspect JIA p-value†	JIA versus other uveitis p-value†	JIA versus control p-value†	JIA N=16	Suspect JIA N=9	Other uveitis N=59	Control N=21	p-value*	JIA versus suspect JIA p-value†	JIA versus other uveitis p-value†	JIA versus control p-value†
4,203						*ND*			0.364	0.233	0.1	0.17	**0.004**	1.000	**0.027**	1.000
4,240						*ND*			0.197	0.12	0.1	0.126	0.12	-	-	-
6,475	0.103	0.234	0.066	0.064	**0.004**	0.738	**0.048**	0.081	0.649	0.405	0.276	0.466	0.242	-	-	-
6,672	0.106	0.211	0.05	0.064	**<0.001**	1.000	**0.003**	**0.03**	1.026	0.518	0.38	0.648	0.164	-	-	-
6,928	0.107	0.109	0.077	0.061	0.118	-	-	-							*ND*	
6,989	0.198	0.125	0.131	0.091	**0.006**	0.399	0.384	**0.006**							*ND*	
7,600						*ND*			0.183	0.143	0.071	0.071	**0.041**	1.000	**0.021**	1.000
7,975						*ND*			0.188	0.2	0.073	0.086	**0.024**	1.000	0.099	0.255
8,161	0.063	0.058	0.1	0.121	**0.001**	0.657	0.072	**0.003**							*ND*	
8,255	0.065	0.063	0.093	0.117	**0.001**	1.000	0.12	**0.012**							*ND*	
8,725	0.096	0.107	0.063	0.061	**0.011**	1.000	**0.024**	**0.012**	0.116	0.06	0.052	0.139	**0.061**	-	-	-
8,840	0.096	0.096	0.068	0.068	**0.035**	1.000	**0.045**	0.117	0.124	0.079	0.051	0.16	**0.005**	1.000	0.126	1.000
8,951						*ND*			0.137	0.073	0.056	0.16	**0.014**	1.000	**0.006**	1.000
9,367						*ND*			0.139	0.066	0.066	0.102	0.213	-	-	-
9,468						*ND*			0.188	0.083	0.062	0.186	0.129	-	-	-
11,742	0.086	0.079	0.115	0.077	0.443	-	-	-							*ND*	
12,869						*ND*			0.092	0.052	0.043	0.069	0.28	-	-	-
13,361	0.109	0.073	0.085	0.112	0.267	-	-	-							*ND*	
13,762	0.232	0.229	0.12	0.087	**0.004**	1.000	**0.045**	**0.021**							*ND*	
13,884	0.397	0.214	0.243	0.139	**0.041**	0.918	0.777	0.063	0.124	0.092	0.063	0.105	0.437	-	-	-
13,914	0.311	0.237	0.199	0.135	**0.008**	1.000	0.351	**0.018**							*ND*	
14,040	0.254	0.231	0.181	0.124	0.064	-	-	-	0.349	0.155	0.115	0.23	0.179	0.349	0.155	0.115
15,214	0.053	0.061	0.084	0.074	0.538	-	-	-	0.304	0.277	0.129	0.124	0.155	0.304	0.277	0.129
15,406	0.043	0.042	0.062	0.074	**0.014**	1.000	0.159	**0.006**							*ND*	
15,874						*ND*			0.278	0.306	0.102	0.118	**0.026**	1.000	0.081	1.000
16,171	0.034	0.037	0.055	0.075	**0.004**	1.000	**0.036**	**0.012**							*ND*	
17,232	0.051	0.05	0.042	0.047	0.647	-	-	-							*ND*	
17,361	0.069	0.078	0.05	0.047	0.183	-	-	-	0.082	0.06	0.044	0.093	**0.013**	1.000	**0.051**	1.000
22,313	0.113	0.108	0.09	0.072	**<0.001**	0.657	**0.03**	**0.003**	0.075	0.061	0.05	0.071	**0.027**	1.000	0.099	1.000
23,262	0.061	0.065	0.066	0.073	0.618	-	-	-							*ND*	
23,824	0.054	0.056	0.06	0.069	0.051	-	-	-							*ND*	
27,981	0.091	0.117	0.051	0.036	**0.018**	1.000	0.285	**0.03**	0.299	0.147	0.103	0.197	0.088	-	-	-
28,122	0.067	0.087	0.049	0.038	0.12	-	-	-							*ND*	
29,004	0.036	0.04	0.037	0.037	0.97	-	-	-	0.103	0.065	0.05	0.071	**0.037**	1.000	**0.048**	1.000
33,410	0.316	0.302	0.222	0.135	**<0.001**	0.738	**0.033**	**0.003**	0.165	0.117	0.09	0.153	0.103	-	-	-

m/z; mass/charge; ND: not detected

P- values are calculated using Mann-Whitney U-test with Bonferroni correction for multiple testing.

* - Mann-Whitney test and Kruskal-Wallis test were used to compare medians between the groups.

† – Bonferroni correction for multiple testing is applied

the AH of JIA- and JIA-suspected uveitis patients, was not detected in serum. Similarly, the AH peaks correlating with the peak at m/z 13,762 (m/z 6,928 and 6,989, and m/z 8,161, 8,255 and 16,171) were not detected in serum (Supplemental Table 1).
Using the presence or absence of AH-specific protein peaks a classification tree was made. Based on the presence of the protein at m/z 13,762 in AH, nine out of 14 JIA patients were clustered as well as three patients with other uveitis entities (two ANA-positive undefined panuveitis and one ANA-positive posterior uveitis with papillitis) were identified incorrectly yielding a sensitivity of 64% and a specificity of 95% (Figure 2). For the calculation of the specificity suspected JIA cases were not taken into account. Adding the protein detected at m/z 8,255, that had the strongest negative expression correlation with the 13,762 peak (Table 4), into the algorithm one incorrectly identified patient with papillitis could be filtered out which increased the specificity of the classification tree to 96%. When applying the algorithm to the AH of patients with suspected JIA-uveitis they were identified with a similar sensitivity as the proven JIA-

Table 4. Bivariate correlations between the expression of protein peaks in the aqueous humor of 14 patients with uveitis associated with juvenile idiopathic arthritis (JIA).

Protein peak, m/z	JIA-uveitis AH		
	Spearman's rho correlation coefficient *		
	6.672	8.725	13.762
6,475	0.763	0.710	NA
6,672	NA	0.767	NA
6,989	NA	NA	0.754
8,161	NA	NA	***-0.745***
8,255	NA	NA	***-0.873***
8,725	0.767	NA	NA
8,840	NA	0.710	NA
13,884	NA	NA	0.780
13,914	NA	NA	0.833
16,171	NA	NA	***-0.723***
27,981	0.547	0.534	0.837

m/z: mass/charge; JIA: juvenile idiopathic arthritis; AH: aqueous humor; NA: not applicable
* Only statistically significant correlation coefficients are presented ($p \leq 0.05$)

uveitis patients (5/8, 63% and 9/14, 64%, respectively; Figure 2). All control patients were clustered separately due to the absence of the peak at m/z 13,762. By multivariate analysis the expression and detection of the peak at m/z 13,762 was associated only with distinct study groups and not with age or duration of uveitis.

To further study the protein profiles in the AH of patients with definitive and suspected JIA a separate automatic peak detection procedure using the same analytical conditions was performed. In this analysis 19 of the initial 26 peak clusters were identified specific for these two groups. An unsupervised heat map for these 19 peak clusters revealed distinct protein expression patterns in samples form patients with active and inactive uveitis independently of definitive or suspected JIA status (Figure 3). The separation between groups based on the protein expression showed major correlation with uveitis activity (Figure 3). All but one inactive JIA and JIA-suspected samples-from a JIA patient with clinically inactive uveitis showed a protein profile which was comparable with active samples (Figure 3). Six up-regulated proteins at m/z 6,928, 6,989, 13,762, 13,884, 13,914 and 14,040 were most clearly associated with uveitis activity (Figure 3). Within the JIA samples no significant differences in protein expression were found regarding type of JIA, ANA serological status, immunosuppressive treatment, presence of cataract, secondary glaucoma, or cystoid macular edema.

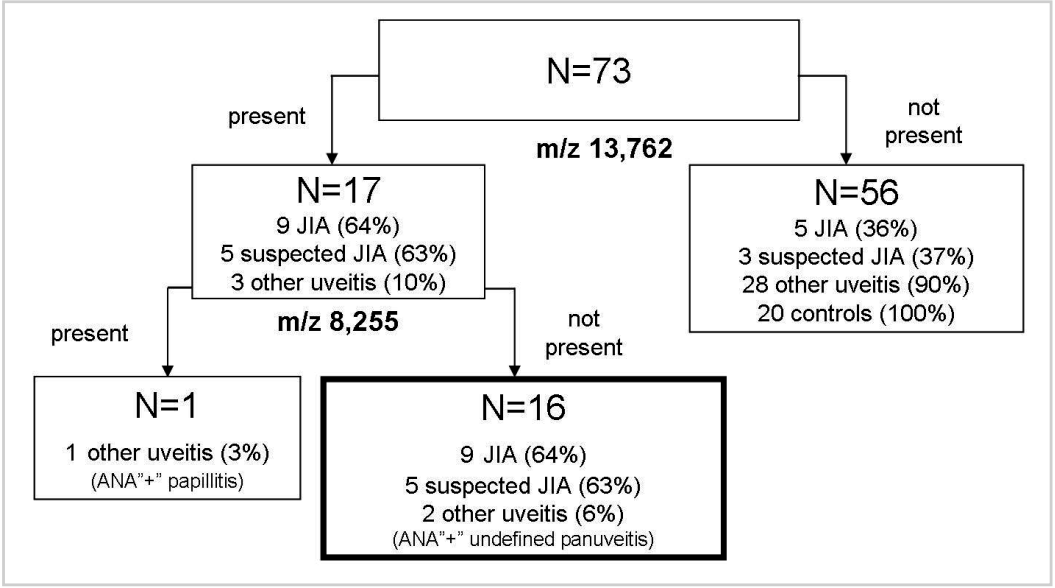

Figure 2. Classification tree to distinguish patients with proven or suspected Juvenile Idiopathic Arthritis associated uveitis from pediatric patients with other uveitis entities and non-inflammatory controls based on the presence of the peak at m/z 13,762 and the absence of the peak at m/z 8,255. The algorithm's sensitivity per group (in percentages) is indicated in the bold lined frame.

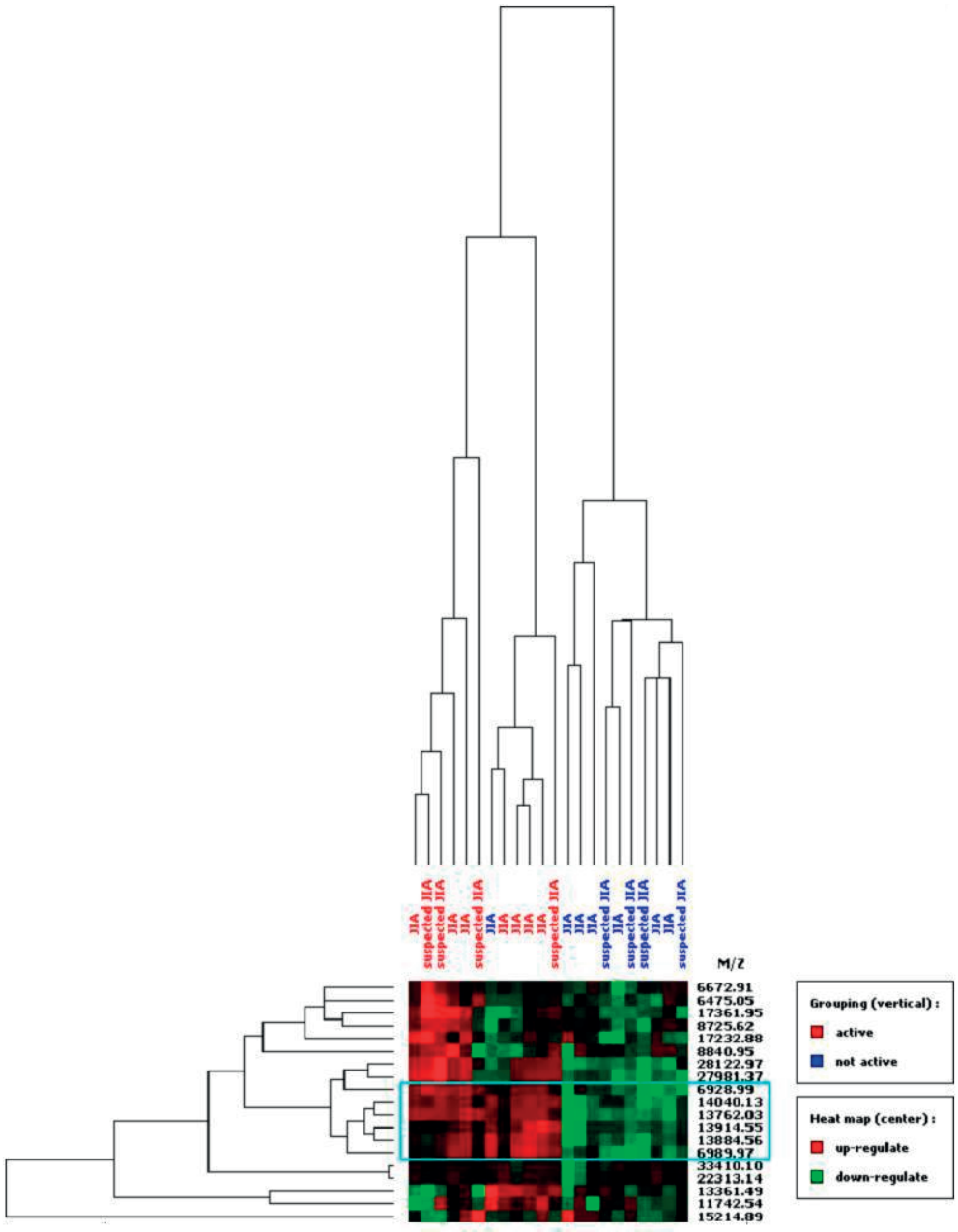

Figure 3. Unsupervised heat-map of protein expression in the aqueous humor (AH) of patients with uveitis-associated with juvenile idiopathic arthritis (JIA) (definitive or suspected) based on 19 peak clusters found in these samples. Active samples are displayed in red, inactive samples in blue. Up-regulation or down-regulation of proteins is indicated on a scale from red to green, respectively. The horizontal axis displays the JIA type, the vertical axis shows the peaks m/z included in the analysis. The proteins most clearly associated with uveitis activity are represented within the blue rectangle.

Biomarker identification

As the target at m/z 13,762 is most indicative for JIA- and suspected JIA-uveitis an attempt was made to purify and identify this protein by LC/MS/MS. Due to the limited volume of AH, all samples of JIA and suspected JIA-uveitis containing the m/z 13,762 peak were pooled and verified by SELDI-ToF MS (Figure 4). As shown in Figure 4 peak clusters at m/z 13,800 and 6,900 were most abundant; the ion population at m/z 6,900 most likely represents the double charged species of the singly charged population at m/z 13,800. Subsequent SDS PAGE resolved several bands within the range of 3 to 20 kDa (Figure 5A). Passive elution of these bands followed by SELDI-ToF MS analysis demonstrated that band 8 contained the target protein at m/z 6,900 and 13,800 (Figure 5B, compare with Figure 4B). LC/MS/MS analysis of the same band (band 8) revealed transthyretin (TTR; Mr 13.76 kDa) as the most abundant protein present in the AH in JIA and suspected JIA samples.

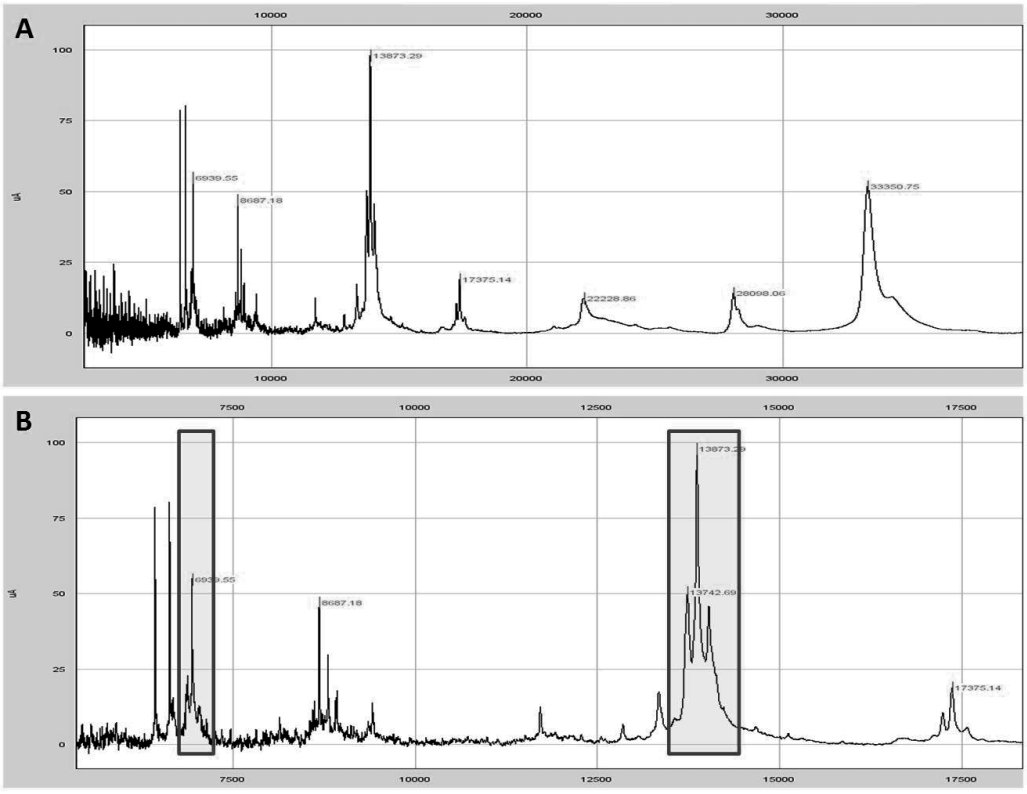

Figure 4. SELDI-ToF MS analysis of pooled aqueous humor (AH) from patients with juvenile idiopathic arthritis (JIA) and suspected JIA uveitis containing the marker at m/z 13,762. (A) Spectrum showing ions detected in the m/z range 2,500-40,000. (B) Close up view highlighting the target ions of interest (blue rectangles). The m/z is represented on the x-axis, whereas the peak intensity is shown on the y-axis.

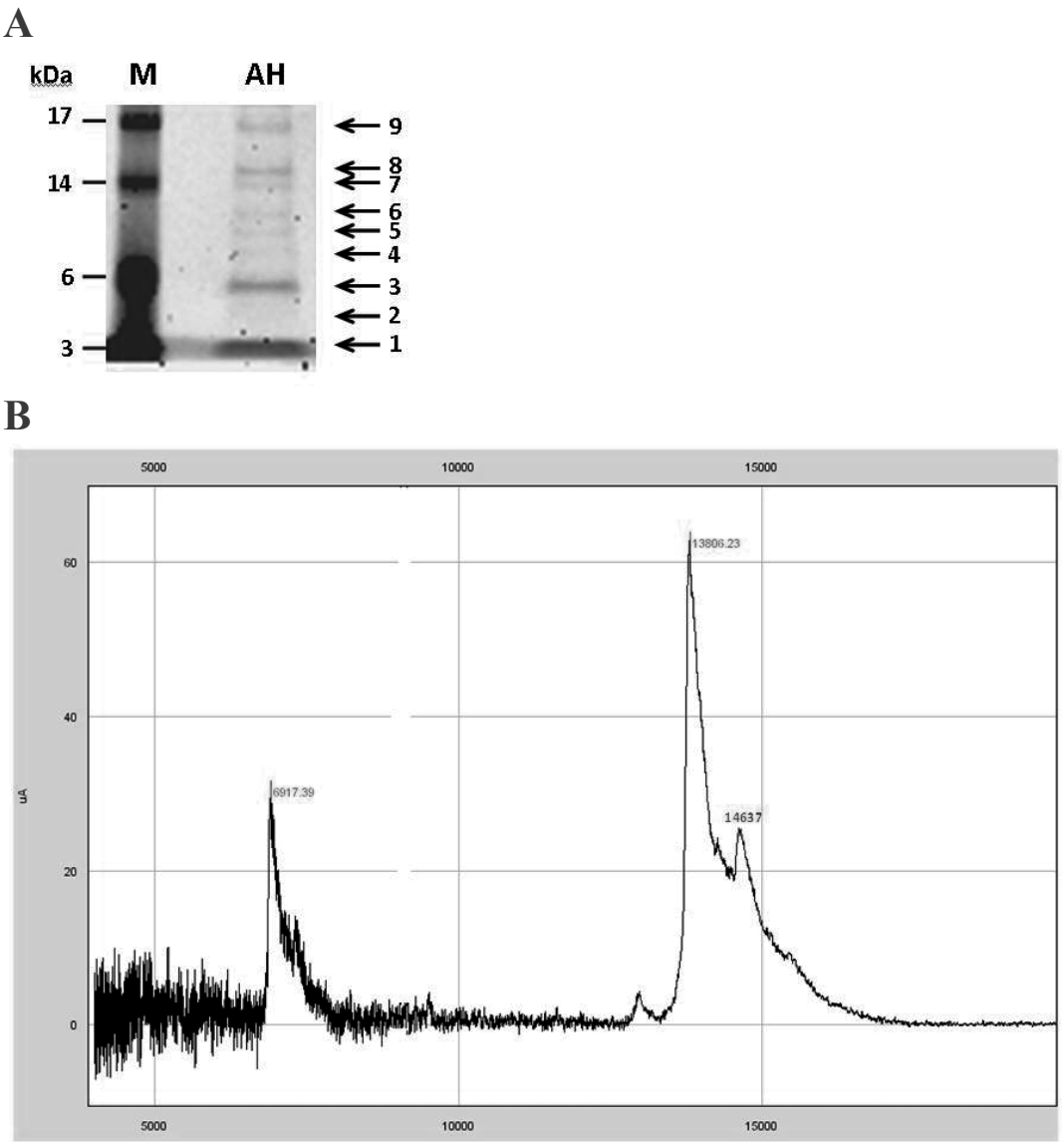

Figure 5. (A). Coomassie-stained 12% 1D SDS PAGE containing 31 mg of pooled aqueous humor (AH) sample (right lane). The molecular weight marker (M) is shown in the left lane. Molecular weights are indicated on the left in kDalton (kDa). The proteins bands analyzed in the AH samples are listed on the right. (B) SELDI-ToF MS spectrum of protein band eight following passive elution from the excised gel slice. The molecular weight in represented on the x-axis, whereas the peak intensity is shown on the y-axis.

Discussion

Our study shows distinct differences in AH protein expressions in JIA-associated uveitis compared to other pediatric uveitis and non-inflammatory pediatric controls. Quantitative expression of JIA-specific proteins was associated with the activity of uveitis.

Classically, the diagnosis of JIA is based on clinical ILAR criteria based on specific rheumatological signs; however a diagnostic test for this disease is lacking. Moreover, the diagnosis of patients presenting with uveitis prior to arthritis is challenging. In our previous work we have described a relatively high percentage of patients who have an atypical manifestation of JIA: with uveitis onset primary to arthritis.[2;3] The diagnosis of JIA in these patients was, in some cases, made several years after the initial onset of uveitis. Our results demonstrate similar protein composition in the AH of patients with definitive JIA-associated uveitis and in patients with putative JIA-associated uveitis presenting with uveitis solely and lacking the rheumatological signs. Further, similar sensitivity for definitive and suspected JIA-associated uveitis was found when the patients were classified according to the classification tree, based on presence of a peak at m/z 13,762. (Figure 2) These findings suggest similar intraocular molecular processes in JIA patients with and without arthritis. The relatively low sensitivity of the classification tree could be explained by an inactive status of uveitis in 50% of (suspected) JIA samples, which seemed to have negative quantitative association with the expression of peak at m/z 13,762 (Figure 3). On the other hand the specificity of the classification method was relatively high, although its calculation can be seen only as estimation due to the absence of a golden standard for the diagnosis of JIA. Interestingly, two patients with other uveitis entities that clustered together with the (suspected) JIA patients had an undefined ANA+ panuveitis without infection or systemic association. One could speculate that in these ANA+ panuveitis cases JIA-association, although not as yet clinically detected, could not be completely ruled out.

In this study TTR was identified as a potential biomarker for JIA-associated uveitis. The nascent protein has a molecular weight of 15.88 kDa. However, as the signal peptide is lost during intracellular protein processing the molecular weight of the mature protein is 13.76 kDa, which is within 0.2% of the observed m/z by SELDI-ToF MS. SELDI-ToF MS analysis of unprocessed AH sample showed that the peak detected at m/z 13.8 kDa resolved into three discernable ions (Figure 4B), which most likely represent different isoforms of the same protein. Indeed, a plentitude of isoforms have been reported for TTR, many of which are causally associated with formation of amyloid

fibrils.[12] Well known systemic functions of TTR are the transport of thyroid hormones and retinol-binding-protein (RBP), however, increasing evidence suggests its involvement in many other biologic processes.[12-14] TTR in the human eye is synthesized by retinal pigment epithelium (RPE) and probably also by ciliary pigment epithelium (CPE), as was recently shown in a rabbit model.[15]. TTR is present in almost all ocular tissues in non-inflammatory eyes, including AH.[16-18] TTR in the vitreous has been demonstrated not to originate from the blood.[17] Similarly, considering its suggested production by CPE, local production of TTR in AH seems plausible. In our study the ion at m/z 13,762 was not detected in serum, which suggests its intraocular origin, although direct comparison of serum and AH spectra by SELDI-ToF MS is probably not accurate.

Our results suggest that TTR may play a role in the pathogenesis of JIA-associated uveitis. The underlying mechanism is unclear; however; one might speculate that the amyloidogenic properties of TTR are involved in secondary membrane and synechiae formation in JIA-uveitis patients. It has been suggested from studies demonstrating TTR production in rabbit CPE cells that TTR expression might contribute to the formation of amyloid fibrils in the pupillary margin and angle chamber.[15] In addition, TTR was reported to be increased in the AH of eyes with myopia and primary open angle glaucoma (POAG) but under-expressed in eyes with primary congenital glaucoma (PCG).[5;7;19-21] Grus *et al*. proposed TTR as a biomarker for POAG, based on the up-regulation of a 14,132 Da TTR species in the AH of POAG eyes.[7] In our study no relationship between POAG, PCG or myopia, and the expression of our target peak at m/z 13,762 was found. These findings suggest the existence of different isoforms of TTR in AH with potentially distinct functions in different (pathological) conditions. Further investigations are required to confirm this hypothesis.

Systemic TTR is known to be a negative acute-phase reactant. Moreover, in endotoxin-induced uveitis (EIU) in a rabbit model, intraocular TTR production by CPE was down-regulated until 48 hours after the induction of EIU.[14] In our study, however, ocular TTR was over-expressed in patients with JIA-uveitis and suspected JIA-uveitis with active disease. This discrepancy can be explained by the fact that inflammation in JIA-uveitis does not display an acute but a chronic process with an insidious onset and a silent course. Most patients with JIA and suspected JIA-uveitis from this study had this type of uveitis for several years prior to AH collection. In addition, the intensity of inflammation in most JIA samples scored as "active" was mild due to their surgical origin. Differences in TTR expression and structure in chronic and acute intraocular inflammation need to be delineated by further research.

Proteomic analysis of synovial fluid in JIA suggests differential expression of proteins based on disease progression and JIA subtype.[22-24] Unlike synovial fluid, AH protein expression profiles did not seem to correlate with JIA subtype, however, the number of patients is too low for a reliable analysis. TTR was not identified in synovial fluid by other studies nor in our SELDI-ToF MS experiments employing synovial fluids from patients with JIA (data not shown). In fact, we could not detect any of the specific peaks detected in the AH of JIA-uveitis patients in synovial fluid in the m/z range 2,500 - 40,000. This suggests local up-regulation of TTR in the anterior chamber in JIA-uveitis without having a systemic effect.

Under normal conditions the protein concentration of AH is low (0.1 – 0.5 mg/ml).[25-27] However, during inflammation the integrity of the blood-aqueous barrier can be severely compromised, causing remarkably increased protein concentrations.[25-27] This can complicate the interpretation of a comparative proteomic analysis of AH between uveitis and control samples. In our study we focused on the proteins of low molecular weight, while especially proteins with high molecular weight seem to accumulate in the case of a compromised blood-aqueous barrier.[25] In the past, AH was considered to be a plasma filtrate,[25;26] however recent studies show the presence of many specific proteins in normal AH.[16;18] Although the functions of the majority of these proteins are not known, their presence suggests active local production by cells and tissues of the anterior segment. Expression levels of AH proteins in our study showed significant correlation with each other which could imply their interactions in the inflammatory or, alternatively, regulatory molecular mechanisms.

To our knowledge this is the first proteomics study on AH of children with uveitis. The strong points of our study are the relatively large numbers of inflammatory and non-inflammatory pediatric AH samples, which allow for reliable statistical analysis and the inclusion of samples from patients with uveitis suspect for JIA-association with incomplete ILAR criteria. Shortcomings are firstly that not in all cases paired AH and serum samples could be analyzed. Secondly, despite a relatively large total number of samples, heterogeneity within the sample groups resulted in lack of power to perform some statistical subgroup analyses, such as JIA entity correlation. Thirdly, due to the practical and ethical difficulty of collecting non-inflammatory ocular fluid in children, it was impossible to match control samples, especially by age and gender. Also the duration of uveitis differed significantly between the groups. However, multivariate analysis adjusted for these differences and showed their insignificance.

As a variety of conditions have been associated with TTR recently, one should question its general value as a biomarker, although specific isoforms or posttranslationally

modified forms of TTR could have specific roles in different (pathologic) conditions. The resolution of the SELDI-ToF MS technique did not allow for the identification of the TTR isoform of relevance in JIA, so further studies into this are warranted.

Another issue is the relatively low detection sensitivity of SELDI-ToF compared with other Shotgun mass spectrometry methods and hence poor analytical depth in terms of how many low abundance proteins can be analyzed.

In summary, AH of patients with JIA-associated uveitis has a distinct expression profile of proteins compared with controls and other uveitis entities. These profiles are comparable with profiles of uveitis patients who on clinical grounds are suspected for JIA-association but do not meet ILAR criteria completely. Expression of these specific proteins within JIA and suspected JIA groups was associated with the activity of uveitis. TTR, the protein identified, could be involved in the pathogenesis of JIA-associated uveitis; however its role and the associated mechanism require further investigation.

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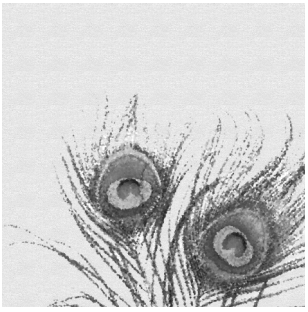

Chapter 8

Immunohistochemical analysis of iridectomy specimens in uveitis associated with juvenile idiopathic arthritis.

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Submitted for publication

Abstract

Purpose: To investigate cellular infiltration in iris biopsies in uveitis associated with juvenile idiopathic arthritis (JIA) in comparison with other pediatric uveitis entities and non-inflammatory pediatric controls.
Design: Interventional case series
Participants and Controls: 30 eyes of 24 patients: 24 eyes with uveitis diagnosed before the age of 16 years and 6 eyes with open angle non-uveitic glaucoma.
Intervention: Iridectomy specimens of iris were obtained during standard trabeculectomy. Histopathologic analysis of all specimens was performed by a haematoxilin and eosin staining. A semiquantitative scoring system was used with a scale ranging from 0 to 4 depending on the intensity of staining of positive cells. In specimens with lymphocyte infiltration scored ≥ 2 and/or in the presence of plasma cells and/or histiocytes additional immunostaining was performed.
Main outcome measure: Histopathologic and immunohistochemical features of JIA-uveitis and other childhood uveitis entities.
Results: Mild to moderate signs of inflammation were present in 7/11 (64%) cases associated with JIA and in 16/24 (67%) of all uveitis specimens. Clinical mild activity of uveitis before surgery was observed only in 1/11 (9%) of JIA and 4/24 (21%) of all uveitis cases. Five different uveitis specimens including JIA, idiopathic anterior uveitis and idiopathic intermediate uveitis, demonstrated CD4+ T cells in the inflammatory infiltrates, while presence of other cell types in these specimens was discrepant. Two JIA-uveitis iris specimens with moderate inflammatory infiltrate showed a non-granulomatous type of inflammatory reaction showed presence of CD4+ , CD68+ and CD138+ cells while CD8+ and CD20+ cells could be detected only in one of them. Another JIA specimen was characterized by the abundance of giant cells typical for a granulomatous process.
Conclusions: CD4+ T cells are present in inflammatory infiltrates in different childhood uveitis entities, including JIA-uveitis. Uveitis in JIA is characterized by a mixed inflammatory infiltrate in the iris with involvement of CD4+ T cells, plasma cells and histiocytes.

Introduction

Uveitis in childhood is a potentially blinding condition with juvenile idiopathic arthritis (JIA) being the most common systemic association in this population.[1] JIA-associated uveitis is characterized by its anterior localisation with iris and ciliary body being primer sites of inflammation.[2-5] Despite intensive research, the pathogenesis of the simultaneous inflammation of eye and joint in JIA remains unknown. From research based on animal models it has been concluded that uveitis is a T cell-mediated disease.[6] However, in humans uveitis is still poorly characterized histopathologically and immunohistochemically due to very limited availability of tissue.. Almost all available histopathological studies of JIA-associated uveitis concern the end-stage of the disease. These case reports suggest a B cell process with heavy infiltration of plasma cells and CD20+ cells in enucleated eyes and sector iridectomy in JIA-associated uveitis.[2-5]
In the present study we provide histopathological and immunohistochemical findings in a relatively large number of iris specimens obtained during therapeutic glaucoma surgery of children with uveitis associated with JIA, other uveitis entities and pediatric non-uveitis controls.

Materials and Methods

Iris specimens ad data collection

Specimens of iris were obtained during standard trabeculectomy with mitomycin with peripheral iridectomy at Department of Ophthalmology, University Medical Center Utrecht, the Netherlands after having obtained a written informed consent from the parents and/or the patients. Specimens were collected from totally 30 eyes of 24 patients: 24 eyes of 19 children with uveitis diagnosed before the age of 16 years and 6 eyes of 5 children with open angle non-uveitic glaucoma. The study was performed, in accordance with the Declaration of Helsinki for research involving human tissue and was approved by the Institutional Review Board.
Diagnosis of uveitis was based on the criteria of the Standardization of Uveitis Nomenclature (SUN) Workgroup Diagnosis of JIA was made by a paediatric rheumatologist according to the criteria from the International League against Rheumatism (ILAR).[8;9]
TE was performed under general anaesthesia by an experienced surgeon specialized in childhood uveitis and pediatric glaucoma (JHB). Trabeculectomy was performed if antiglaucomatous therapy (local) had failed to control intraocular pressure and/or

increased pathological optic disc cupping and/or progression of visual field defects. It is generally approved to perform intraocular surgery when the eye is quiet for at least 3 months preoperatively; however because of the urgent indication in some cases, TE was performed in eyes with mild uveitis activity shortly before surgery. Activity of uveitis was scored according to the grading system advocated by the SUN working group.[7] All patients with uveitis were pre-treated with systemic corticosteroids (1 mg/kg) starting 2 days prior surgery. Children with non-uveitic glaucoma had no history of intraocular inflammation.

The following clinical data were collected from patients' medical records: demographics, date of onset of uveitis, anatomical type and etiology of uveitis, previous intraocular surgery, treatment of uveitis at the moment of the sample collection and eventual recent activity of uveitis preoperatively.

Histopathological and immunohistochemical investigation

All specimens were fixed in 10% formalin, embedded in paraffin and stained with haematoxylin and eosin (HE) to evaluate the following histological features: (1) the presence of lymphocyte infiltration; (2) the presence of plasma cells; (3) the presence of histiocytes (4) fibrosis of iris stroma; and (5) the presence of hypervascularization. The sections were viewed by light microscopy at magnification 400x by an independent observer who was not aware of the diagnosis. A semiquantitative scoring system was used with a scale ranging from 0 to 4 depending on the intensity of staining of positive cells. A score of 1 represented mild infiltration, while a score of 4 represented infiltration by numerous inflammatory cells. In specimens with moderate signs of inflammation, defined as lymphocyte infiltration ≥ 2 and/or the presence of plasma cells and/or histiocytes, additional immunostaining was performed. The primary antibodies used were CD3 antibody to detect the presence of T lymphocytes, CD4 for T helper cells; CD8 for cytotoxic T cells, CD20 for B lymphocytes, CD68 for histiocytes and CD138 to identify the presence of plasma cells. Immunohistochemical sections were examined under a light microscope at 400x magnification.

Results

Histopathological findings

Table 1 presents the summary of the demographics, clinical and histopathological

characteristics of the study specimens. All but 2 uveitis specimens were obtained under chronic administration of systemic immunosuppressive therapy with methotrexate being the main used agent (regardless the standard pre-treatment with systemic corticosteroids 2 days before surgery in uveitis patients) (Table 1).

Histopathological findings in uveitis cases

Mild to moderate signs of inflammation were present in 7/11 (64%) cases associated with JIA and in 9/13 (69%) of other uveitis specimens. Clinical mild activity of uveitis before surgery was observed only in 1/11 (9%) of JIA and 4/24 (21%) of all uveitis cases. Figure 1 presents typical histopathologic findings in selected JIA and non-JIA specimens. The lymphocyte infiltration was most pronounced in case 2 (JIA; Figure 1 A), 12 (anterior uveitis; Figure 1 B) and 22 (intermediate uveitis) (Table 1). In 2/24 uveitis specimens, plasma cells could be identified (case 1 (JIA; figure 1C) and 14 (anterior uveitis)). In this JIA specimen (case 1) additionally to plasma cells also histiocytes were identified. Case 3 showed pronounced granulomatous inflammation characterized by the abundance of histiocytes with giant cells and collagen destruction. (Figure 1D).
Fibrosis was present in 3 uveitis specimens (case 3, 9 and 12; Table 1). Seven out of 24 iris samples of uveitis patients with different diagnoses showed hypervascularisation (Table 1).

Histopathological findings in control cases

Mild lymphocyte infiltration was found in 2 paired iris specimens with congenital glaucoma (case 25, 26). Three out of 6 non-uveitic glaucoma iris specimens showed fibrosis and 4/6 hypervascularisation. None of the control iris samples showed pronounced presence of inflammatory cell infiltrate, plasma cells and/or histiocytes.

Immunohistochemical findings

Results of the additional immunostaining of iris in 5 cases with moderate inflammatory infiltrate are presented in Table 2. Figure 2 shows the results of immunohistochemical staining in two JIA specimens. In both ANA+ JIA-uveitis iris specimens CD4+ T cells were accompanied by varying numbers of CD68+ and CD138+ cells (Figure 3 and 4). CD8+ cells and CD20+ cells were present only in one of the two JIA specimens (case 1) (Figure 2). All 5 immunohistochemically stained iris specimens with different uveitis entities showed CD4 positivity, while only two of them were also CD8 positive (case 1 (JIA; Figure 2) and 12 (anterior uveitis ANA-)); Table 2). Totally 3 cases showed also

Table 1. Patient demographics, clinical characteristics and histopathological findings in iris and trabecular meshwork biopsies

Case No.	Age (years)	Sex	Diagnosis	Duration of uveitis (years)	No. of surgery	Anterior chamber cells pre-op[a]	Systemic therapy[b]	Topical steroids	Hematoxillin & eosin staining[c]	
									Grade inflammatory cell infiltration[d]	Fibrosis, hyperemia
Uveitis										
1	6.0	F	JIA-uveitis ANA +	2.7	1	-	MTX	Yes	Lymphocytes 1, plasma cells 1, histiocytes 1	Hypervascularisation
2	13.5	F	JIA-uveitis ANA +	9.2	1	-	MTX	Yes	Lymphocyte 2	-
3*[e]	4.9	F	JIA-uveitis ANA -	1.8	1	-	MTX	Yes	Histiocytes 3	Fibrosis
4*	5.0	F	JIA-uveitis ANA -	1.9	1	-	MTX, Prednison	-	-	-
5	15.2	F	JIA-uveitis ANA +	10.4	4	-	Adalimumab Azathioprin	Yes	Lymphocytes 1	-
6	7.6	F	JIA-uveitis ANA +	2.9	1	-	MTX	Yes	Lymphocytes 1	-
7	9.1	F	JIA-uveitis ANA +	8.1	1	trace	MTX	Yes	Lymphocytes 1	-
8*	23.9	F	JIA-uveitis ANA +	3.8	1	-	MTX, Adalimumab	-	Lymphocytes 1	-
9*	26.3	F	JIA-uveitis ANA +	4.7	1	-	MTX, Adalimumab	Yes	-	Fibrosis, hypervascularisation
10	5.2	F	JIA-uveitis ANA +	2.9	1	-	MTX, Adalimumab	Yes	-	-
11	11.1	F	JIA-uveitis ANA +	8.1	1	-	-	Yes	-	-
12*	10.6	M	Anterior uveitis ANA -	3.8	1	trace	MTX	Yes	Lymphocytes 2	Fibrosis, hypervascularisation
13*	11.5	M	Anterior uveitis ANA-	4.7	3	-	MTX	Yes	Lymphocytes 1	-
14	5.3	M	Anterior uveitis ANA +	1.5	1	trace	MTX	Yes	Plasma cells 1	Hypervascularisation
15*	9.7	F	Anterior uveitis ANA +	0.9	1	-	MTX	Yes	Lymphocytes 1	Hypervascularisation
16*	10.0	F	Anterior uveitis ANA +	4.8	1	-	MTX	Yes	Lymphocytes 1	-

17	10.6	F	Anterior uveitis ANA +	2.8	1	-	MTX	Yes	-	-
18	9.2	F	Anterior uveitis ANA -	1.5		trace	MTX	Yes	-	-
19	8.0	F	Anterior uveitis ANA -	4.3	1	-	MTX	Yes	-	-
20*	9.7	M	Anterior uveitis ANA -	1.8	1	-	MTX	-	Lymphocytes 1	-
21*	9.8	M	Anterior uveitis ANA -	1.9	1	-	MTX	Yes	Lymphocytes 1	Hypervascularisation
22	8.8	M	Intermediate uveitis	0.7	1	-	MTX	-	Lymphocytes 2	-
23	9.6	M	Intermediate uveitis	2.6	1	-	-	-	Lymphocytes 1	Hypervascularisation
24	10.8	M	Panuveitis	2.1	1	trace	MTX	Yes	-	-
Control										
25*	0.3	M	Congenital glaucoma		1	-	-	-	Lymphocytes 1	Hypervascularisation
26*	0.3	M	Congenital glaucoma		1	-	-	-	Lymphocytes 1	Hypervascularisation
27	0.4	M	Rieger syndrome, glaucoma		1	-	-	-	-	Fibrosis, Hypervascularisation
28	1.1	M	Congenital glaucoma		1	-	-	-	-	Fibrosis, Hypervascularisation
29	2.7	M	Congenital glaucoma		1	-	-	-	-	Fibrosis
30	0.1	M	Neurofirbromatosis, glaucoma		1	-	-	-	-	-

* Indicated paired eyes;

Pre-op, preoperative; JIA, Juvenile Idiopathic Arthrits; MTX, Methotrexate;

[a] According to the grading system advocated by the Standardization of Uveitis Nomenclature (SUN) working group.[7]

[b] Regardless standard pre-treatment with systemic corticosteroids 2 days before surgery

[c] Only positive findings according to the semiquantitative scoring system are noted

[d] A semiquantitative scoring system was used with a scale ranging from 0 to 4 depending on the intensity of staining of positive cells. A score of 1 represented mild infiltration, while a score of 4 represented infiltration by numerous inflammatory cells.

[e] No material was available for additional immunohistochemical stainings

a variable number of CD20+ cells (Table 2). In case 14 (ANA+ anterior uveitis) these cells were located in a distinct cluster, while in the other specimens these cells showed a diffuse infiltrating pattern.
CD68+ cells were identified in variable numbers in case 1, 2 (Figure 3) and 22 (Table 2). The presence of CD138+ cells marked both ANA+ JIA cases (case 1 and 2; Figure 3) and ANA+ anterior uveitis (case 14) (Table 2). In case 3 with granulomatous inflammation characterized by the abundance of histiocytes with giant cells no additional material was available for immunohistochemical staining.

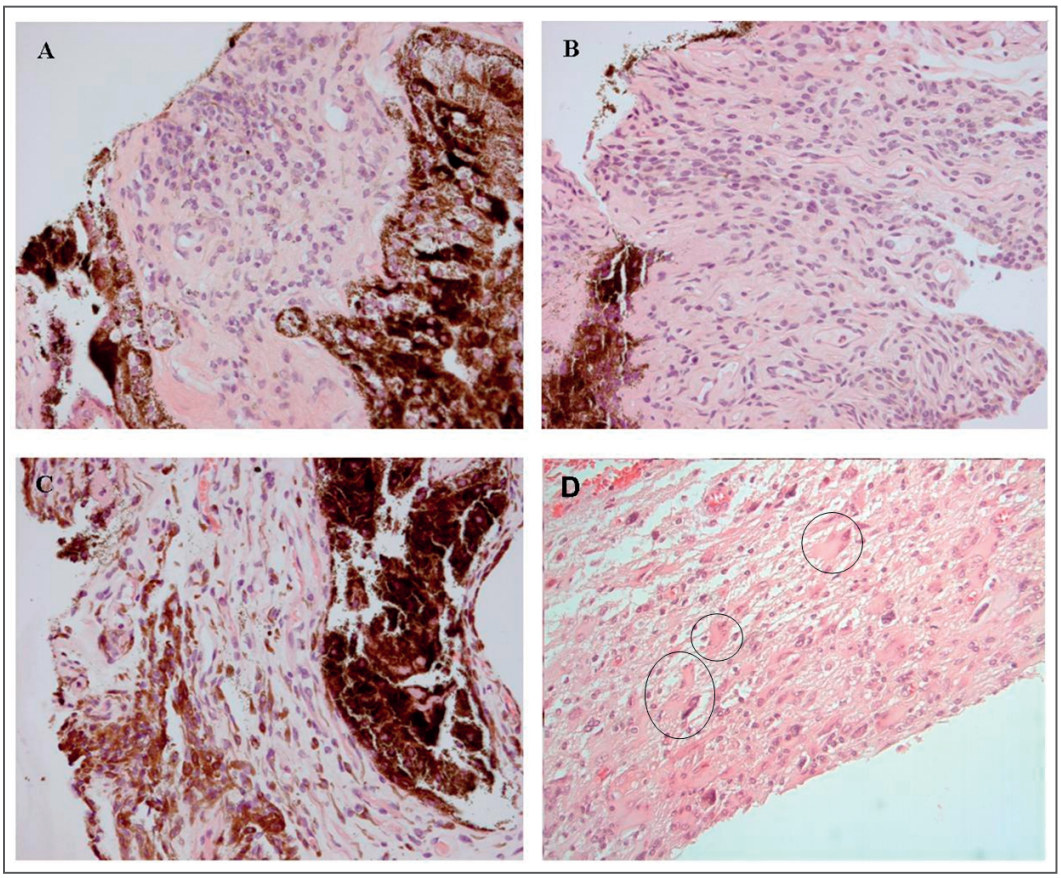

Figure 1: Photomicrographs showing the histopathologic results from the iridectomy specimens obtained during selective trabeculectomy in children with anterior uveitis associated with juvenile idiopathic arthritis (JIA) and without identified systemic association. Magnification 10x20. Haematoxillin and eosin staining A. Case 2 (JIA). B. Case 12 (ANA-negative anterior uveitis). C. Case 1 (JIA). D. Case 3 (ANA-negative JIA). A and B show moderate non-granulomatous inflammation. C shows mild non-granulomatous inflammation. D shows a picture typical for granulomatous inflammation characterized by the presence of giant cells (marked with circles).

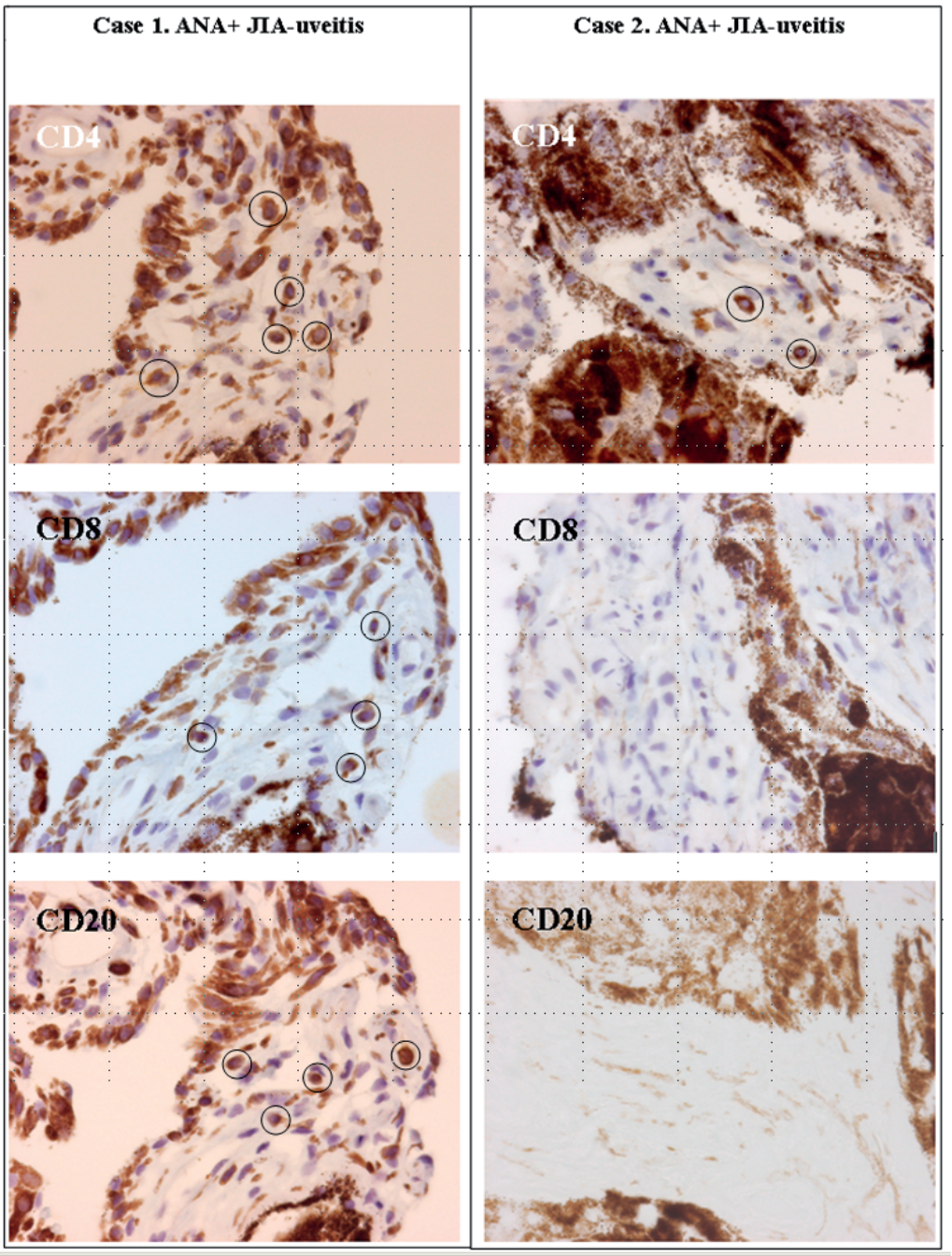

Figure 2. Photomicrographs showing the results of immunohistochemical analysis of two iridectomy specimens obtainded during elective trabeculectomy in two patients with uveitis associated with juvenile idiopathic arthritis (JIA). The results of CD4, CD8 and CD20 analysis are shown. Positive cells are marked with circles. Magnification 10x40.

Table 2. Results of immunohistochemical staining of iridectomy specimens in 5 cases with more pronounced presence of inflammatory cells in hematoxilin & eosin staining.[a]

Case No.	Type of uveitis	ANA	Age (years)	Age of uveitis onset (years)	Age of arthritis onset (years)	Duration of uveitis (years)	CD20	CD3	CD4	CD8	CD68	CD138
1	Persistent oligoarticular JIA, RF "-"	+	6.0	3.3	1.5	2.7	2	2	3	2	3	3
2	Persistent oligoarticular JIA, RF "-"	+	13.5	4.3	4.0	9.2	-	-	2	-	1	2
12	Anterior uveitis	-	10.6	6.8	NA	3.8	-	1	2	2	-	NM
14	Anterior uveitis	+	5.3	1.5	NA	3.8	2[b]	1	2	-	NM	2
22	Intermediate uveitis	-	8.8	8.1	NA	0.7	1	2	2	-	1	NM

[a] In case 3 no material was available for additional immunohistochemical staining.
[b] focal arrangement
NA, not applicable; NM, no material available for analysis

Table 3. Overview of previously published histologic reports on ocular specimens form patients with uveitis associated with juvenile idiopathic arthritis.

Year	Authors	Specimen	ANA	Age of diagnosis of uveitis (years)	Duration of uveitis	Histological type of inflammation	Predominant inflammatory cells in the iris	Antibody
1979	Sabates et al.	Enucleation Enucleation	NR NR	3.5 9.5	16 years 7 years	Non-granulomatous Granulomatous(?)*	Plasma cells[a] Lymphocytes, plasma cells, giant cells[a]	NR NR
1981	Godfrey et al.	Iridectomy	+	4	7 months	Non-granulomatous	Plasma cells[a]	IgM
1983	Merriam et al.	Enucleation	+	3	7 years	Non-granulomatous	Plasma cells, lymphocytes[a]	IgG >IgM
2008	Parikh et al	Enucleation	+	4	7 years	Non-granulomatous	Plasma cells, CD20+ B-lymphocytes, T cells mostly CD8+[b]	IgG > IgM and IgA

[a] Histopathologic examination
[b] Histopathologic and immunohistochemical examination
* Several giant cells were seen, however, in the study of Parikh et al (2008) where a similar histopathologic picture was seen, immunohistochemical analysis revealed that the cells mimicking giant cells were ciliary epithelial cells. NR: not reported

Discussion

To our knowledge this is the first histological study of iridectomy specimens from patients with JIA-associated uveitis, compared to other pediatric uveitis entities and non-inflammatory controls. It is noteworthy that despite a quiescent clinical state of uveitis in the majority of the cases, some of them still had significant infiltration of inflammatory cells in the iris. The cellular infiltration seemed to have no clear relation to the clinical activity of uveitis. A common nonspecific finding in the uveitis specimens was the presence of CD4+ T cells in the mixed inflammatory infiltrate.

Although our immunohistochemical results in JIA encompass only two cases, CD4+ T cells were present in both specimens whereas CD8+ T cells only in one of them. CD4+ T cells are thought to play the most prominent role in the pathogenesis of arthritis in JIA and also experimental uveitis models emphasize the essential role of CD4+ in contrast to CD8+ cells in the induction of non-infectious uveitis in mice.[6;10] Inflammation in these autoimmune disorders is considered to be a disbalance between the pathogenic and regulatory subsets of CD4+ T cells: T helper 1 (Th1) and T helper-17 (Th17) and regulatory T cells (Tregs).[6;10]

Plasma cells, the final differentiation stage of B-cells, were relatively abundant in both JIA specimens. These findings are consistent with earlier histological studies on enucleated eyes and one iridectomy in JIA-uveitis which report plasma cells to be the most prominent cell type infiltrating the iris, suggesting an important role of B cells in the pathogenesis of JIA-uveitis.[2-5] Table 3 summarizes the findings of the previous reports on this subject. So it seems that in both earlier and end-stage of inflammation, plasma cells take the prominent place in the inflammatory infiltrate. As has been shown earlier, they actively produce immunoglobulins (Table 3).[2-4] Specimens with abundant plasma cells in our study originated from ANA-positive patients, however unfortunately no material was available in ANA-negative patient for additional anti-CD138 staining. Whether these plasma cells actually produce autoantibodies at the site of ocular inflammation can not be elucidated from our study.

Additionally to plasma cells and CD4+ cells also CD68+ cells were in variable numbers present in both JIA specimens. CD68 is a general histiocyte marker which is though unable to distinguish between macrophages and dendritic cells.[13] Despite the common origin and shared markers these cells represent two discrete cell types with distinct functions: phagocytosis (macrophages) and antigen presentation to T cells (dendritic cells). So, CD68+ cells can represent a hallmark of the activation of both innate and adaptive immune systems, which are in fact intertwined and both involved in the

pathogenesis of autoimmune diseases, including non-infectious uveitis and JIA.[10;14] It has also been reported that many other non-myeloid cells can express CD68 on their surface, for instance, fibroblasts.[15] However, histopathological examination did not show signs of fibrosis in these specimens. Because of very limited material it was impossible to further specify CD68+ cells in our specimens, which can be a focus of interest in the future research.

To our knowledge there was until now only one report with immunohistochemical analysis in JIA-uveitis. Parikh et al describes the findings in an enucleated eye after 7 years of disease (Table 3).[4] Results of our study show that CD4+ cells infiltrate the iris

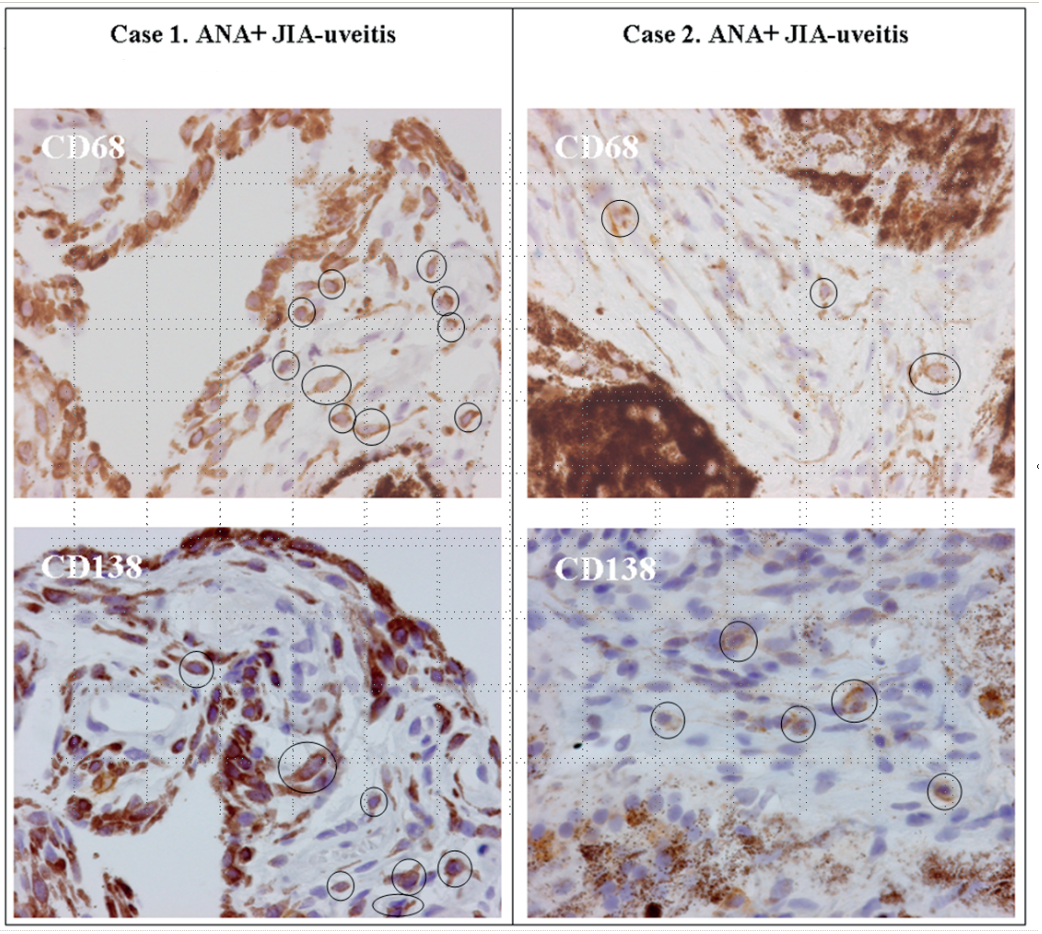

Figure 3. Photomicrographs showing the results of immunohistochemical analysis of two iridectomy specimens obtainded during elective trabeculectomy in two patients with uveitis associated with juvenile idiopathic arthritis (JIA). The results of CD68 and CD138 analysis are shown. Positive cells are marked with circles. Magnification 10x40.

in JIA-uveitis. This in contrast to the study of Parikh et al, where CD4+ were seen only occasionally and mainly not in the iris. This could be explained by the fact that Parikh et al describe an end-stage histological picture in an enucleated eye while our results represent eyes with less deleterious consequences of chronic inflammation. Also differences in treatment, including the use of anti-TNFα agents in the study of Parikh et al could potentially declare the low numbers of CD4+ cells. Our other findings, especially regarding infiltration of plasma cells, are in accordance with the study of Parikh et al.

Histopathological picture of the one of JIA-specimens with clinically non-granulomatous ANA-negative uveitis was characterized by the presence of giant cells showing a typical picture of a granulomatous inflammation. This is a remarkable finding as uveitis in JIA is generally considered as a non-granulomatous entity.[2-4] Sabates et al also reported presence of epithelioid cells and giant cells in a clinically non-granulomatous JIA-uveitis.[5] However, as has been shown later, in an absence of immunohistochemical analysis ciliary epithelial cells can be misinterpreted as epitheliod and giant cells in these specimens.[4] Although, morphologically the cells infiltrating the iris with possibly granulomatous inflammation looked as typical histiocytic giant cells and not as epithelial cells, we are aware of this possible misinterpretation and regret that no additional material of this iris was available for immunohistochemical analysis in our study. It is noteworthy that another study suggested that clinically granulomatous uveitis can be found in children with JIA more frequently (28%) than previously reported.[16]

Although this research provides some new insights in the histopathological picture of JIA-uveitis and childhood uveitis in general, we are aware of its limitations and shortcomings. Because of the surgical origin of the specimens, most of the patients had clinically quiet chronic uveitis and showed absent to mild inflammatory infiltrate in the iris. This resulted in a small number of specimens suitable for detailed immunohistochemical examination. Due to the low number and heterogeneity of these specimens no proper comparison between the entities was possible. A very small size of the specimens limited the possibilities of histopathologic and immunohistochemical analysis. Although it was not an initial goal of our study, it would be interesting to further specify the subset of the CD4+ T cells and CD68+ cells detected in the inflammatory infiltrates, which was also unfeasible in the current report, but should be the focus of a new study with a larger number of samples. A larger multicenter study would increase the power of the analysis and make better comparisons possible, for instance between ANA+ and ANA- patients.

In summary, this report shows the presence of CD4+ T cells in inflammatory infiltrates in different childhood uveitis entities, including JIA-uveitis. Despite the mixed cellular infiltrate with discrepant composition, the presence of CD4+ T cells, CD68+ and plasma cells was similar in two analyzed JIA-cases. These findings could form be the basis for further research for the specification of the subsets and the roles of these cells in the pathogenesis of JIA-associated uveitis.

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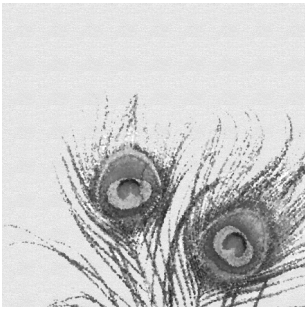

Chapter 9

Young age as a risk factor for complicated course and visual outcome in intermediate uveitis in children

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Abstract

Objective: To identify prognostic factors in intermediate uveitis (IU) in children.
Methods: Retrospective case series of 35 patients with onset of IU <16 years and a minimum follow-up of 1 year. Demographic and numerous clinical data were documented. Visual outcomes and development of complications were analysed in relation to age of onset and ocular signs at presentation.
Results: Forty-six per cent of patients had onset ≤7 years, and 54% >7 years. The younger-onset group had a shorter event-free survival for secondary glaucoma (p = 0.04) and vitreous haemorrhage (p = 0.01). The mean age of onset in children with cataract (5.9 vs 8.7 years), glaucoma (5.0 vs 8.4) and vitreous haemorrhage (5.6 vs 8.5) was lower than in children without these complications (all p = 0.03). Frequencies of other complications did not differ between both groups. The younger-onset group had worse BCVAs at presentation (0.3 vs 0.6), at 1 year (0.4 vs 0.9) and at 3 years' follow-up (0.6 vs 0.9; all p ≤ 0.04), and they needed longer treatment (p = 0.01). Children with young onset of IU reached remission less frequently (p = 0.05). Development of cystoid macular oedema was independently associated with papillitis (adjusted HR = 3.4; p = 0.02) and snowbanking (adjusted HR = 3.3; p = 0.03) at presentation. Other complications at onset were not predictive for future complications.
Conclusions Children with young onset of IU carry a higher risk of complications and worse visual outcome. The authors would recommend considering more intensive monitoring and earlier threshold for systemic treatment in those children with risk factors as early onset, papillitis and/or snowbanking at initial presentation.

Introduction

Intermediate uveitis (IU) occurs in up to 42% of the paediatric uveitis population and is the second most common form of uveitis in childhood.[1] Idiopathic IU is characterised by a chronic inflammatory process primarily involving the vitreous, posterior ciliary body and peripheral retina.[2] Pars planitis (PP) is a form of idiopathic IU characterized by snowballs and snowbanking at the pars plana and peripheral retina in the absence of systemic diseases.[2]

IU in adults can occasionally be associated with systemic disorders or infections; however, in most children the aetiology of IU remains unknown. The understanding of the pathogenesis of IU is still limited; involvement of auto-immune processes and/or a genetic predisposition[3-7] has been suggested, and several familial cases have been reported.[8-12]

The course of IU in children can be worsened by many sight-threatening complications.[13-19] Nevertheless in many children and young adults IU can reach remission with maintained good visual acuity in the absence of ongoing therapy; their disease can resolve and 'burn itself out'.[14;19;20] Although the visual outcome in many children is favourable, approximately 19% develop unilateral legal blindness.[14]

One of the major questions in IU is whether it is possible to predict which patients are at greater risk of developing severe course of IU with visual loss before the sight-threatening complications actually occur. It has been reported that children with IU might have a more severe disease and worse outcome than patients with onset at older age,[21-23] but these findings could not be confirmed in other studies.[17;19]

Patients and Methods

Medical charts of all paediatric patients with idiopathic IU examined at the department of Ophthalmology, University Medical Center, Utrecht, The Netherlands, were reviewed. The patients were collected from the database of our institution between 1989 and 2009. All 35 patients with the onset of their IU before the age of 16 years and a minimum follow-up of 1 year were included in this study. Diagnosis of IU and PP was made in agreement with the criteria of the Standardization of Uveitis Nomenclature (SUN) workgroup.[2] Combination with anterior segment inflammation was allowed.

All children were evaluated by a paediatrician and/or paediatric immunologist before

the idiopathic nature of IU was concluded. Numerous data from the medical charts of the patients were recorded, and demographics, date of diagnosis of IU, laterality of ocular disease, extent of diagnostic procedures and duration of follow-up were noted. The presence and date of onset of all ocular signs and complications were noted. Owing to difficulties in objective measurement of cataract formation, we have used cataract surgery as a measure for cataract. Secondary glaucoma was defined as the presence of pathological cupping of the optic disc and/or a glaucomatous visual-field defect, in combination with intraocular pressure (IOP) higher than 21 mm Hg or both.[24] Cupping of the optic disc was defined as pathological when there was a C/D difference of >0.2 between fellow eyes or when an increase in cupping was noted compared with the previous visit. Cystoid macular oedema (CMO) was defined as the presence of macular thickening with cyst formation seen by funduscopy and/or by optical coherence tomography. Vitreous involvement was classified into two categories: presence of cells in vitreous or presence of dense vitreous opacities (in addition to cells). Vitreous opacities were quantified according to the recommendations of the SUN working group by slit-lamp examination for the anterior vitreous and funduscopy of the posterior vitreous.[2] Vasculitis was scored if vascular sheating/leakage was observed on funduscopy and/or angiography. The duration of uveitis was calculated from the date of diagnosis of IU. All surgical procedures and pharmacological treatment options (with initiation and withdrawal date) were registered as well. Remission of IU was defined as the absence of inflammatory activity in both anterior chamber and vitreous, and no signs of snowballs and/or snowbanking for at least 1 year in the absence of anti-inflammatory therapy. There were no children who were lost to follow-up within the first year after the discontinuation of the therapy.

In addition to examination by an ophthalmologist specialized in childhood uveitis, all children were examined by an orthoptist at initial visit and regularly evaluated during the follow-up. None of the study population had causes of amblyopia other than IU. Standard frequency of follow-up examinations was approximately once every 2-3 months.

Patients were subdivided according to age at onset of IU into a younger-onset (IU onset ≤7 years of age) and older-onset group (IU onset >7 years of age). This approach was chosen to allow bivariate comparisons and survival analysis between the groups. Best-corrected visual acuities (BCVAs) were scored at onset of IU and during the follow-up. Snellen BCVAs were converted into the logarithm of the minimum angle of resolution scale (logMAR) for statistical analysis and converted back to Snellen BCVAs for data presentation.[25] Legal blindness was defined as BCVA of the affected eye of

20/200 or worse.[26] In the analysis of visual outcome, only the eyes with vision loss due to uveitis or related complications were included. Visual acuities were analysed by eye (with an adjustment for paired eyes), and further data were analysed by patient.[27] Statistical analysis of the data was performed with SPSS 15.0.1. The Pearson X^2 or Fisher exact test was used for univariate analysis of categorical variables. The means were compared using the Student t test for normally and Mann-Whitney U test for abnormally distributed variables. Kaplan-Meier survival analysis with a logrank test was used to analyse survival and to compare groups. Cox proportional hazard regression was applied for multivariate analysis of variables significant in univariate analysis. BCVAs were analysed using linear regression with GEE as adjustment for paired eyes.[26] p Values of ≤0.05 were considered statistically significant. For presentation, we used means if data were distributed normally (by Kolmogorov-Smirnov test) and medians if it was not. All significances are two-tailed and nominal.

Results

General characteristics of the study group and subgroups according to the age of IU onset are presented in table 1. Thirty-one patients (89%) fulfilled the criteria of PP,[2] and four remaining patients did not show the presence of snowballs and/or snowbanking during their follow-up. In 10/35 (29%) patients, diagnosis of IU was made during routine ophthalmological examination without ocular complaints, and the others were referred to an ophthalmologist because of an ocular complaint of floaters or decreased visual acuity.

Table 1 General characteristics and therapy of patients with idiopathic intermediate uveitis according to age of uveitis onset

Characteristics	Total, N = 35	Age of uveitis onset		p Value
		0–7 years, N = 16 (%)	7–16 years, N = 19 (%)	
Female gender (N; %)	10 (29)	4 (25)	6 (32)	0.72
Bilateral disease (N; %)	33 (94)	16 (100)	17 (90)	0.49
Follow-up years (median; range)	4.0 (1.3–16.9)	4.3 (2.4–16.7)	4.0 (1.3–14.1)	0.22
Topical steroids (N; %)	30 (86)	16 (100)	14 (74)	0.05
Periocular steroid injections (N; %)	24 (69)	12 (75)	12 (63)	0.45
Systemic corticosteroids (N; %)	12 (34)	7 (44)	5 (26)	0.28
Immunomodulatory therapy (N; %)*	5 (14)	4 (25)	1 (5)	0.16

*More than one immunosuppressive agent was subsequently used in three patients (methotrexate (n=4), ciclosporin (n=2), azathioprine (n=1) and mycophenolate mofetil (n=1)).

Ocular signs and complications at onset of IU (initial presentation)

The ocular findings at the initial presentation for the whole group, and in the groups divided according to the age of onset, are presented in table 2. It is notable that moderate to dense vitreous opacities[2] were significantly more frequently present in children with onset 7 years (88% vs 42%; p = 0.01). At the initial orthoptic examination, amblyopia due to IU was present in four patients (11%), and in one of them the diagnosis of IU was made during treatment for amblyopia (table 2).

Ocular signs and complications in the course of follow-up

Ocular signs and complications in the course of follow-up and mean time until their development are listed in table 3. The most frequently observed complications were papillitis (60%) and CMO (57%), which were bilateral in the majority of cases (table 3). Children from the onset group ≤7 years developed severe sight-threatening complications such as secondary glaucoma and vitreous haemorrhage (p = 0.04, p = 0.01, respectively; figures 1 and 2) earlier and more frequently. Additionally, the mean age of onset in children in whom the course of IU was complicated by cataract surgery, secondary glaucoma and vitreous haemorrhage was significantly lower than in children without this complication (respectively, 5.9 vs 8.7; 5.0 vs 8.4 and 5.6 vs 8.5 years; all p = 0.03).

Development of CMO in the course of follow-up was univariately associated with the presence of papillitis and snowbanking at onset (both p < 0.01). In a multivariate analysis, adjusted for age of uveitis onset and gender, the presence at onset of both papillitis (adjusted HR = 3.4; 95% CI 1.1 to 9.6; p = 0.02) and snowbanking (adjusted HR = 3.3; 95% CI 1.2 to 9.7; p = 0.03) were independently associated with later development of CMO. Other complications at onset were not predictive for future complications.

Treatment

All but one patient (97%) in our series needed some form of anti-inflammatory therapy during follow-up (table 1). Topical steroids (86%) and periocular steroid injections were used most frequently in all patients (86% and 64%, respectively). Systemic medication in the form of systemic corticosteroids and immunosuppressives was administered in 34% and 14% of the patients, respectively. Table 1 demonstrates that patients with young onset seemed to be treated more intensively in our series. However, this difference reached the level of statistical significance only for the use of topical steroids (100% vs 68%; p = 0.02).

Over time, anti-inflammatory therapy (topical and systemic) could be stopped compl-

Table 2 Frequency of ocular signs and complications at initial presentation in children with intermediate uveitis according to the age of onset

Complications and ocular findings at onset of intermediate uveitis	Total, N = 35 (%)	Age of uveitis onset		p Value*
		0–7 years, N = 16 (%)	7–16 years, N = 19 (%)	
Vitreous opacities (moderate to dense)† (N; %)	22 (63)	14 (88)	8 (42)	0.01
Anterior chamber inflammation (N; %)	11 (31)	6 (38)	5 (26)	0.48
Cystoid macular oedema (N; %)	10 (29)	4 (25)	6 (32)	0.72
Papillitis (N; %)	9 (26)	5 (31)	4 (21)	0.70
Snowballs (N; %)	6 (17)	2 (13)	4 (21)	0.67
Snowbanking (N; %)	6 (17)	2 (13)	4 (21)	0.67
Amblyopia (N; %)	4 (11)	4 (25)	ND	NC
Posterior synechiae (N; %)	2 (6)	2 (13)	ND	0.20
Vasculitis (N; %)	2 (6)	ND	2 (11)	0.49
Epiretinal membranes (N; %)	2 (6)	1 (6)	1 (5)	1.00
Bandkeratopathy (N; %)	1 (3)	1 (6)	ND	0.46
Cataract (N; %)‡	1 (3)	1 (6)	ND	0.46
Vitreous haemorrhage (N; %)	1 (3)	1 (6)	ND	0.46

*p values are computed with Fisher exact test.
†According to the Standardization of Uveitis Nomenclature classification.[2]
‡In one child, diagnosis of intermediate uveitis was made directly after cataract extraction.
NC, not comparable; ND, not detected.

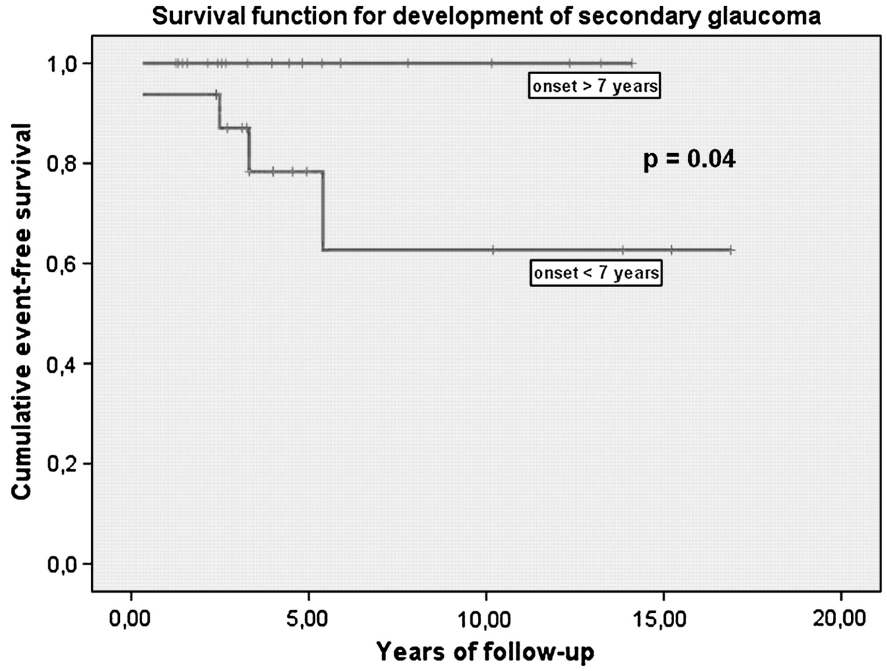

Figure 1. Survival plot for secondary glaucoma in patients with onset of intermediate uveitis (IU) before and after 7 years of age.

etely in 24/35 patients (69%). Fifteen of these 24 patients (63%) reached remission. The mean time to discontinuation of therapy was 2.0 years (range 0.1-6.9 years). The mean age of discontinuation of therapy was 11.0 years (±3.1 SD; range 6.3-18.2 years).
Continuation of any kind of therapy (topical, systemic or periocular) was significantly longer and more frequently needed in children with a younger age of onset compared with the olderonset group (p = 0.01; figure 3).
Pars plana vitrectomy (PPV) was performed in three (9%) children in cases of vitreoretinal traction, and all of them had onset of IU before 7 years of age.

Remission

In total, 15 patients (43%) from the whole group (n = 35) reached remission. The mean time before reaching the remission was 5.1 years (±3.2 SD; range 1.1-11.8). Two of these 15 patients (13%) who reached remission had a flare-up of uveitis after approximately 2 and 2.5 years of remission, respectively. The other 13/15 patients (87%) stayed in remission until the end of follow-up (mean duration of follow-up in remission 3.9 years ±2.9 SD; range 1.1-9.8 years). The mean age of reaching the remission was 13.8 years (±3.1 SD; range 8.1-19.4). In the group with onset of IU ≤7 years, remission was reached in 4/16 patients (25%) versus 11/19 (58%) patients with older onset of IU (p = 0.050).

Visual outcome

BCVAs in affected eyes according to the age of onset of IU at different points of FU are

Table 3 Frequency and time of development of complications, during the follow-up of children with intermediate uveitis according to the age of onset

Complications and ocular findings during the course of intermediate uveitis	Total (N; %), N=35	Bilateral (%)	Age of uveitis onset		Mean time to development (years±SD)
			0–7 years (N; %) N=16	7–16 years (N; %) N=19	
Papillitis (N; %)	21 (60)	90	10 (63)	11 (58)	0.7 (±1.1)
Cystoid macular oedema (N; %)	20 (57)	70	10 (63)	10 (53)	0.6 (±1.3)
Vasculitis (N; %)	16 (46)	63	8 (50)	8 (44)	1.8 (±1.8)
Cataract surgery (N; %)	9 (26)	22	7 (44)	2 (11)	2.6 (±2.1)
Secondary glaucoma (N; %)	4 (11)	ND	4 (25)	ND	2.9 (±2.1)
Vitreous haemorrhage (N; %)	6 (17)	17	6 (38)	ND	3.2 (±2.9)
Bandkeratopathy (N; %)	6 (17)	17	5 (31)	1 (5)	3.2 (±2.4)
Amblyopia (N; %)	6 (17)	ND	6 (38)	ND	0.5 (±1.2)
Posterior synechiae (N; %)	5 (14)	20	3 (19)	2 (11)	0.7 (±1.1)
Epiretinal membranes (N; %)	5 (14)	40	4 (25)	1 (5)	1.9 (±2.1)
Retinal detachment (N; %)	3 (9)	ND	2 (13)	1 (5)	1.2 (±1.4)
Retinal neovascularisation (N; %)	2 (6)	ND	ND	2 (11)	0.6 (±0.6)

ND, not detected.

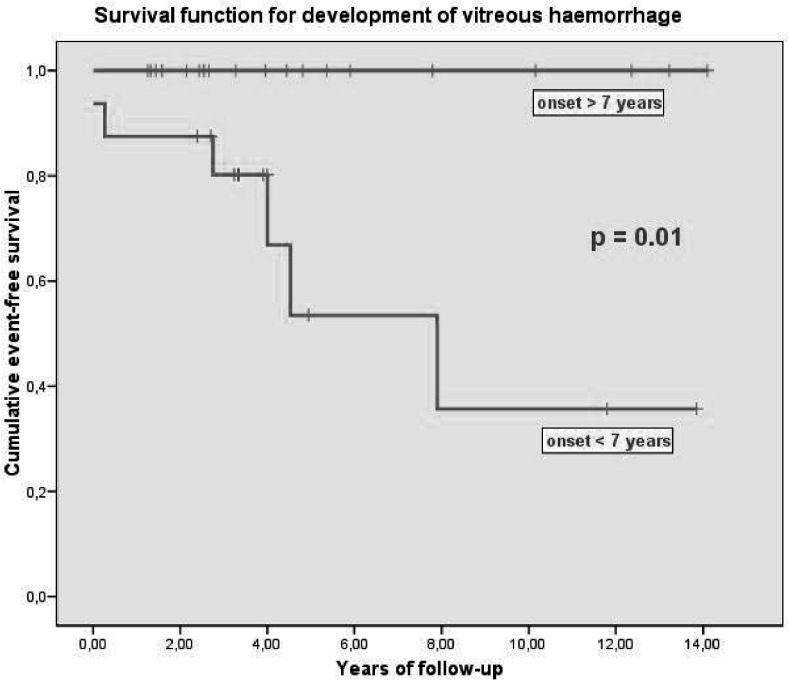

Figure 2. Survival plot for vitreous haemorrhage in patients with onset of intermediate uveitis (IU) before and after 7 years of age.

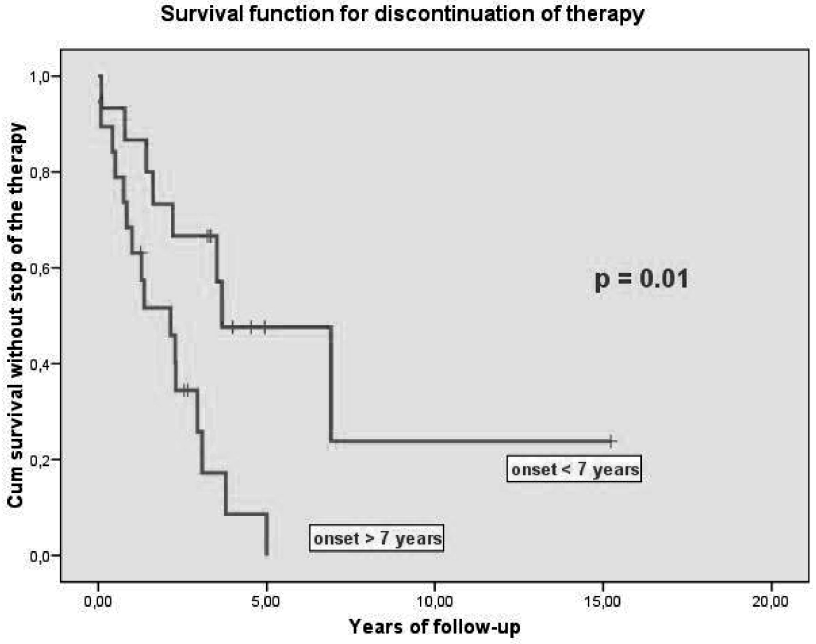

Figure 3. Survival plot for discontinuation of the therapy in patients with onset of intermediate uveitis (IU) before and after the age of 7 years.

presented in table 4. Children with onset of IU ≤7 years had significantly worse BCVAs up to 3 years of follow-up compared with the older-onset group (all p ≤ 0.04; table 4). Unilateral legal blindness occurred in 7/35 patients (20%). Two of them (2/7; 29%) already had one legally blind eye at the initial visit to an ophthalmologist for IU, with no improvement after treatment. Blindness was univariately associated with diagnosis made at routine ophthalmological examination without ocular complaints (p = 0.04). There were no statistical differences in the frequency of blindness between younger- (4/16; 25%) and older-onset groups (3/19; 16%).

The causes of unilateral blindness included CMO/maculopathy (n = 3), retinal detachment after vitrectomy due to epiretinal membranes (n = 2), recurrent vitreous haemorrhage (n = 1) and secondary glaucoma (n = 1). The child with recurrent vitreous haemorrhage was not followed in our clinic anymore since these events. Data on his later visual function were unavailable. In 2/7 (29%) unilaterally blind patients, amblyopia due to IU played a role in the definitive loss of vision.

Discussion

The current study shows a negative effect of young age at onset of IU on the clinical course and visual outcome up to 3 years' follow-up.

To our knowledge, this is the first study to show the effect of young age of onset within a paediatric population with IU. A previous study on paediatric IU from our centre had a different main goal and methodological approach with a slightly different patient selection and reported no differences at presentation between different age groups.[14] In the current study, we estimated the differences in the course of disease and visual outcome between younger and older onset of IU in childhood. It should be mentioned that in accordance with our earlier results, no differences in complications at presentation between different age groups were found.

Our study revealed that children with earlier onset of IU are at increased risk of vitreous haemorrhage, secondary glaucoma and cataract surgery. Vitreous haemorrhage in IU is usually caused by retinal neovascularisation.[16] The fact that vitreous haemorrhage was more common in children than in adults was reported previously by others.[16] It is noteworthy that even within a paediatric population, we found a higher prevalence of vitreous haemorrhage in children with onset ≤7 years of age. In our series, vitreous haemorrhage took place only in children with onset before the age of 7. The difference in prevalence of this complication in children with younger and older

onset could be explained by a more severe form of disease with increased incidence of retinal neovascularisation or by late identification and treatment of neovascularisation in young children. Unfortunately, these data do not make it possible to investigate these theories, as in our series, neovascularisation was observed only in two patients with older onset, and none of them developed vitreous haemorrhage during their follow-up. This example illustrates the difficulty in identification of neovascularisation in young children, as angiography and pars plana indentation are performed mostly in older patients.

A worse visual outcome of children with younger onset of IU could be theoretically explained by a higher frequency of vision threatening complications. Noteworthy is that children with a younger onset had a lower visual acuity at presentation. The speculative explanation for this difference could lie in the difficulty and delay of diagnosis of IU in young children, who mostly do not complain about a decrease in vision. Vitreous opacities, which were more frequent in young children at presentation, could indicate more severe disease at presentation and lead to worse vision at presentation. An additional potential risk and danger in this group of young children is the development of amblyopia, which, as we show in our series, can occur in a sizeable proportion of patients and contribute to their poor visual outcome.

Although almost all patients in our series needed some form of treatment during their disease course, the therapy could be completely stopped in almost 70% after the mean interval of 2 years. In accordance with earlier results from our centre, we show the therapy-free remission of over 1 year in more than a third of patients.[14] An interesting finding of this study is that children with a younger onset of IU needed more prolonged and intensive treatment, which could be discontinued less frequently, and their disease burned out significantly less often. The mean age when remission was reached was 13.8 years, and none of the patients reached remission before the age of 8 years. These facts could indirectly indicate the severity of disease in younger children. As discussed above, children of a young age at onset were at increased risk of complications despite the fact that they received a more intensive treatment. On the one hand, the given treatment might have been insufficient for their severe inflammation, but on the other hand secondary glaucoma and cataract can also be associated with administration of corticosteroids. It is not possible to make a reliable distinction between a more severe inflammation and more intensive use of corticosteroids as a cause for these complications in our data set. More frequent use of topical steroids in children with young onset could be related to a more frequent involvement of the anterior chamber. However, this difference did not reach the level of statistical significance in

our series at onset. The presence of anterior segment inflammation later in the follow-up was not scored.

Papillitis and CMO were the most frequent complications of IU, which is in accordance to other reports.[14;18;23;28] We confirmed the clinical suspicion about the association of these two complications[14] by showing that the presence of papillitis at onset is an independent prognostic factor for development of CMO later. We consider this a clinically relevant finding, since CMO is known as a major cause of poor visual prognosis in these patients[14;20;28] and is being used as a treatment indication.[14;28] Development of CMO was also independently associated with the presence of snowbanking at initial presentation. The knowledge of early risk factors for CMO might help in the identification and the evaluation of patients who are at risk of having this sight-threatening complication.

Several limitations should be mentioned for this study. First, the retrospective study design makes it impossible to account for all possible confounders and complicates the interpretation of cause and consequence. Cataract surgery was chosen as a measurement for cataract. Although this is not a completely objective measurement, in a retrospective study it solves the problem of frequently incomplete documentation of cataract development and observations by different ophthalmologists during the follow-up. As the study was performed in a tertiary centre specialised in uveitis, the milder and uncomplicated cases might be lost to follow-up earlier, so the cumulative complication rates can be overestimated due to a selection bias. The relatively limited number of patients with the small number of events increases the likelihood of type II error (inability to confirm significant difference when it actually exists). The chosen cut-off point of 7 years is arbitrary. It was chosen because it is generally assumed that children under this age have a greater chance of developing amblyopia. The choice of this cutoff point resulted in two statistically comparable groups. Furthermore, the higher complication rate in young children is confirmed by the analysis of age as a continuous variable in children with and without complications.

In conclusion, our study emphasises the increased risk of a complicated and more prolonged course of disease and worse visual outcomes in children with a younger onset of IU. We show that the presence of papillitis and snowbanking at onset of IU is associated with later development of CMO. Our results could help a clinician to identify children with IU who carry a higher risk of complications and worse visual outcome. We would recommend considering more intensive monitoring and an earlier threshold for systemic treatment in those children with risk factors as early onset, disc oedema and/or snowbanking at initial presentation.

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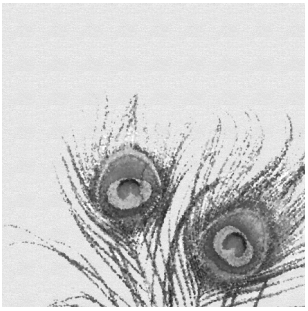

Chapter 10

Intermediate uveitis and alopecia areata: is there a relationship? Report of 3 pediatric cases

Viera Kalinina Ayuso
Jan Willem Pott
Joke H. de Boer

Pediatrics 2011; 128(4):1013-1018

Abstract

Three previously healthy children, aged 5, 8, and 15 years, with idiopathic intermediate uveitis (IU) and alopecia areata (AA) are described. These are the first 3 cases of which we are aware with this coexistence. The results of extensive diagnostic evaluations were negative in all 3 cases. AA preceded the diagnosis of bilateral IU in 1 child and followed within several months after IU diagnosis in 2 children. The severity of uveitis ranged from mild to sight-threatening, and hair loss ranged from local lesions in 2 cases to total alopecia in 1 case. Pathogenesis of both diseases is discussed. Theoretically, the coexistence of IU and AA might be based on the similarities in their complex pathogenesis. However, more research is needed to evaluate if the coexistence is based on an association between 2 autoimmune disorders or is a coincidence.

Introduction

Intermediate uveitis (IU) is a subset of uveitis that is characterized by the primary site of intraocular inflammation located in vitreous. It is the second most common form of uveitis in childhood.[1] In most cases in children, no underlying disease can be found.[1–3] The course of IU in children can be worsened by sight-threatening complications such as cystoid macular edema (CME) and papillitis.[2–4] Although the visual outcome in many children is favorable, up to 20% of patients can develop unilateral loss of vision.[3,4]

Alopecia areata (AA) is a chronic inflammatory disorder of the hair follicles that results in nonscarring patchy hair loss of the scalp. In some patients it can progress to a total loss of scalp hair (alopecia totalis). The incidence of AA is about 0.2%, and the lifetime risk is 1.7%.[5] There is no gender predisposition, and people of all ages can be affected;[5] however, onset during childhood and adolescence is most common.[6]

To our knowledge, a combination of idiopathic IU and AA has not been previously described.

Patients and Methods

Our patients visited the department of ophthalmology at University Medical Center Utrecht between 1997 and 2010. Informed consent was obtained. The diagnosis of IU was made in agreement with the criteria of the SUN workgroup by an ophthalmologist who specializes in (pediatric) uveitis (Dr. de Boer).[7] All children were evaluated by a pediatric rheumatologist and/or immunologist before the idiopathic nature of IU was concluded. The diagnosis of AA was made by a dermatologist. This study was approved by the institutional review board of Utrecht Medical Center.

Case reports

Case 1

A well 8-year-old white girl presented with reduced vision in her left eye. Her medical history included atopy, erythema nodosum at the age of 5 years with no systemic trigger identified, and AA of her scalp at the age of 7 years in the absence of other systemic disorders. Her family history was negative for AA but did include cutaneous lupus in

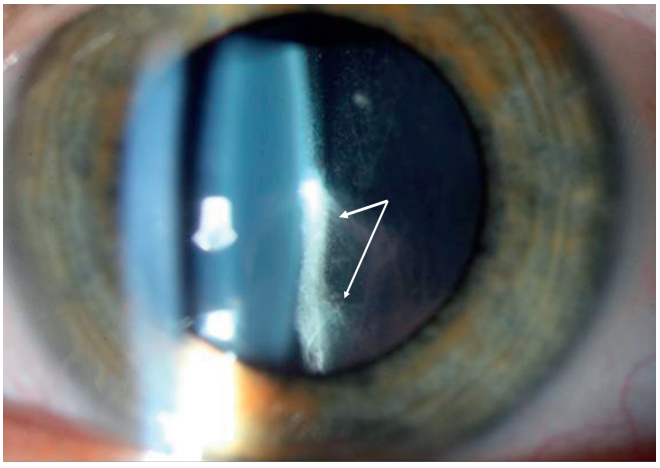

Figure 1. Dense vitreous opacities at the moment of diagnosis in case 1.

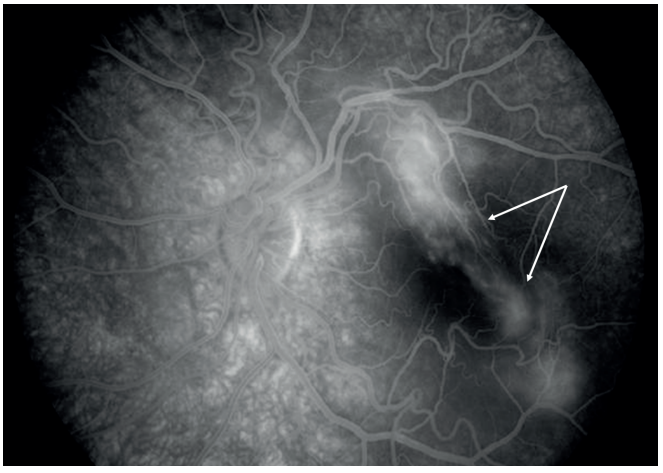

Figure 2. Fluorescein angiogram that shows traction with secondary CME in case 1.

TABLE 1 Values of Selected Immunoserological and Biochemical Parameters From a Diagnostic Workup in 3 Pediatric Patients With Coexistence of AA and IU

Selected Laboratory Parameters	Normal	Case 1	Case 2	Case 3
Angiotensin-converting enzyme, U/L	7–20	17	20	14
Lysozyme, mg/L	6.0–12.0	NT	8.6	7.8
Antinuclear antibodies	Negative	Negative	Negative	Negative
Anti–double-stranded DNA, IU/mL	0.0–15	0.40	Negative	NT
Rheumatoid factor, IU/mL	0–20	<20	Negative	NT
p-ANCA	Negative	Negative	Negative	Negative
c-ANCA	Negative	Negative	Negative	Negative

p-ANCA indicates protoplasmic-staining antineutrophil cytoplasmic antibodies; c-ANCA, cytoplasmic-staining antineutrophil cytoplasmic antibodies; NT, not tested.

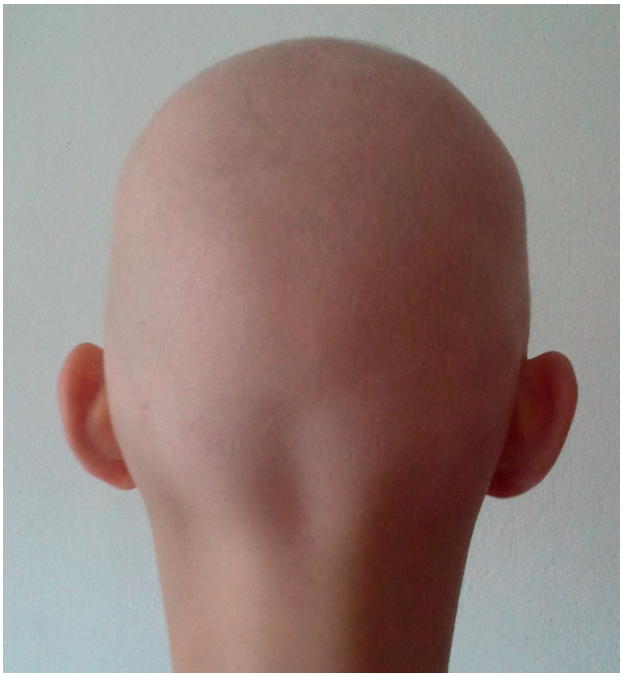

Figure 3. Total alopecia in case 1.

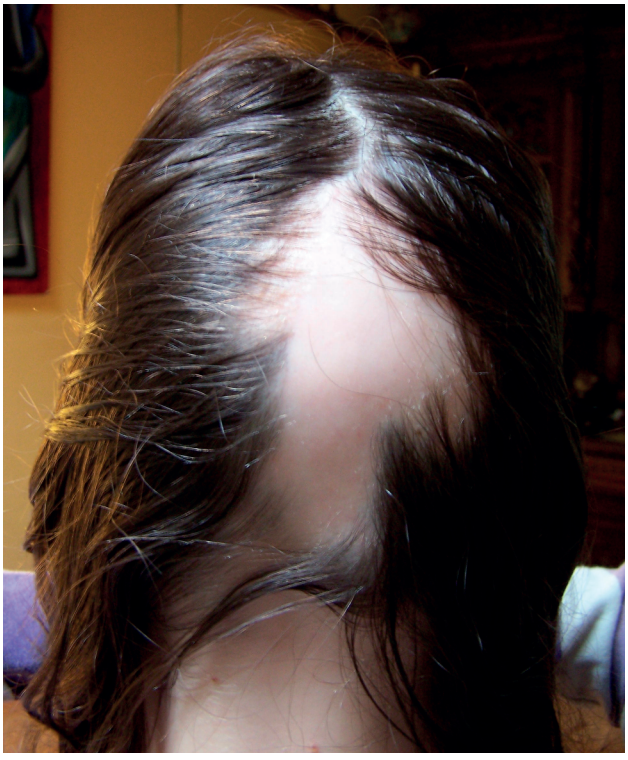

Figure 4. Alopecic lesion in case 2.

2 aunts. Alopecia preceded the onset of visual complaints by 1 year and was treated with local corticosteroids with no effect. No other medications were used.
Bilateral IU was diagnosed. Her Snellen visual acuity was 1.0 in the right eye and 0.2 in the left eye. Both eyes had severe inflammation and opacities[7] in the vitreous (Fig 1). With fundoscopy the left eye showed the presence of papillitis, CME, and peripheral alterations of pigment epithelium; in the right eye, hyperemic optic disks and snowballs (conglomerated vitreous opacities) were seen. No serous subretinal fluid was present. Aqueous tap results were negative for cytomegalovirus, herpes simplex virus, varicella-zoster virus, and rubella virus. An extensive workup including laboratory tests of hematologic, biochemical, and immunoserological parameters, thyrotropin, and urine, the Mantoux test, infection serology (including *Treponema pallidum*, *Borrelia,* and *Bartonella*), chest radiography, chest high-resolution computed tomography, and an abdominal ultrasonography did not reveal any significant disturbances (Table 1). Analysis of T and B cells revealed normal results. No clues for systemic disorders associated with her uveitis and/or alopecia could be found. She was initially treated with periocular steroid injections.
Three months later her visual acuity in the left eye decreased to 0.1 as a result of severe CME and epiretinal membrane formation (Fig 2). Treatment with periocular steroid injections, systemic steroids, and acetazolamide did not improve the CME, but the uveitis became inactive. Vitrectomy in the left eye was performed. The vision in her left eye improved significantly and was 0.7 six months after surgery. Vision in her right eye was 0.9. The uveitis stayed in remission in the absence of medication.
Her hair loss had a progressive course and evolved to a total alopecia within 2 years after the onset (Fig 3). No other dermatologic abnormalities were present.

Case 2

A 5-year-old white girl was seen in our clinic for a second opinion. At the age of 4 years she was referred to an ophthalmologist after decreased vision in both eyes was identified at a periodic medical screening of schoolchildren. Bilateral IU was established by an ophthalmologist. Her medical history was unremarkable. She was using no medication. Her grandmother suffered from scleroderma.
Her vision was 0.3 in both eyes. Vitreous showed the presence of inflammatory cells and dense opacities attached to the lens capsule in both eyes. Fundoscopy results were unremarkable. Several months later her parents noticed an alopecic lesion at the back of her head (Fig 4). Results of an extensive diagnostic workup including hematologic,

biochemical, and immunoserological parameters (Table 1), infection serology (including *Treponema pallidum, Borrelia,* and *Bartonella*), the Mantoux test, and HLA typing were negative for systemic diseases. The patient was treated with topical steroids.
After worsening of her cataract, cataract extractions with intraocular lens implantations were performed in both eyes, 3 and 6 years after the onset of uveitis. Afterward, her Snellen vision was 0.63 in the right eye and 1.0 in the left eye.
Her alopecia remitted after several years; however, it relapsed again soon and evolved to a total alopecia with loss of all scalp hair. Treatment with local steroids had no effect on the alopecic lesion. No other dermatologic abnormalities were present.

Case 3

A 15-year-old white boy presented to our clinic with coexistence of bilateral IU and AA at the back of his head. Onset of uveitis took place at the age of 12 years. Alopecia commenced 1 year later. No other dermatologic or neurologic abnormalities were present. His medical history was negative for systemic disorders. Family history was positive for diabetes, asthma, and cardiac problems.
His IU had a mild course and did not require treatment initially. Later, topical steroids were administered intermittently. Despite the 3-year history of uveitis, good visual acuity was maintained: 1.0 in the right eye and 0.9 in the left eye. Slit-lamp examination revealed vitreous cellular infiltrate in the right eye and sporadic vitreous cells in the left eye. With fundoscopy hyperemic optic discs and peripheral vasculitis were seen. Snowballs and snowbanking were present in the right vitreous. Serous subretinal fluid was absent.
Results of repeated evaluations for systemic disease, including hematologic, biochemical, and immunoserological parameters, were negative (Table 1). Thyroid and adrenal function were normal. Infection with *Bartonella, Borrelia, Toxoplasma, Treponema pallidum* and *Mycobacterium tuberculosis* was excluded. The results of highresolution computed tomography of the chest, lung function testing, and a urine test were unremarkable.
Two years later the patient had a visual acuity of 0.7 in the right eye and 0.9 in the left eye, and there was presence of sporadic cells in the vitreous in absence of therapy for approximately 2 years.

Discussion

We describe here 3 pediatric cases with coexistence of idiopathic IU and AA in the absence of a systemic disease. To our knowledge, this association has not been reported before in the literature.

Clustering of different autoimmune diseases and their higher prevalence in families have been reported.[6;8–10] In our series, all 3 children had other autoimmune conditions in their family history.

The only known association of uveitis and alopecia is the Vogt-Koyanagi-Harada syndrome. This syndrome results from a T-cell–mediated autoimmune attack on melanocytes and usually affects people with greater skin pigmentation between the ages of 20 and 50 years.[11] Onset in childhood is rare and is described only among the genetically susceptible pigmented population.[11] Our cases reveal a distinct clinical picture from a classic Vogt-Koyanagi-Harada syndrome.[12]

Several ocular abnormalities that occur in patients with AA have been described previously. Lens abnormalities and cataracts are being reported most frequently, followed by pigmentary disturbances of the choroid, retina, and iris.[13–16] A case with an optic neuropathy in a child with AA was recently described.[17] No association with uveitis was identified.[14]

The exact pathogenesis of IU and AA has not yet been revealed. Recent studies have found evidence for an autoimmune basis for both conditions.[6;18–23] Both conditions are T cell mediated, and T helper (Th) type cells play a major role in the pathogenesis.[6,18–23] Although in the more extensive form of AA the immunity seems to be polarized toward a Th1 response, in IU a Th1/Th2 bias is less clear, but most data also suggest a Th1 polarization.[18–22;24] In both diseases, interferon γ, tumor necrosis factor α, interleukin 2, and interleukin 6 seem to play a role in the pathogenesis.[18–20;22–24] A genetic predisposition seems to be important for the susceptibility; numerous HLA class I (AA) and II (AA, IU) and other gene (AA) associations have been proposed.[6;22;23;25–28] As with many other autoimmune conditions, the risk of development might be determined by a complex combination of genetic and environmental factors. For AA, stress seems to play a role in disease development, at least in some patients, and there is a potential impact of neuroendocrine factors such as substance P.[6;18;22;23] The involvement of substance P in the pathogenesis of uveitis has been shown in experimental animal models.[29]

Although both disorders are considered to be autoimmune-mediated, no autoantigens have been identified yet.[18;22;25;30] Clinical and histologic observations in AA suggest the

involvement of melanocyte-derived epitopes in this autoimmune process; however, the results of the experiments are controversial.[22;30] The observed ocular pigmentary changes in patients with AA could support this hypothesis.[13,14]

Both the eye and the hair follicle are considered to be immune-privileged sites with similar mechanisms of immune privilege.[6;18;22;29;31;32] So, an immune-privilege collapse or an escape from peripheral tolerance might be key factors associated with the induction of both conditions.[22;30;31]

Our institutional database contains 340 children with uveitis, including 40 children with idiopathic IU; 3 of them have AA, whereas no one from the other group of 300 children with other uveitis entities suffered from AA during our follow-up. This results in a prevalence of AA of 7.5% within idiopathic IU cases, which is higher than the prevalence of 1% to 2% in the healthy population.[5]

Conclusions

Theoretically, the coexistence of IU and AA might be based on the similarities in their complex pathogenesis; however, more research is needed for confirmation.

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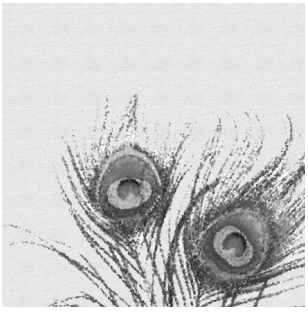

Chapter 11

The effect of an Ahmed glaucoma valve implant on corneal endothelial cell density in children with glaucoma secondary to uveitis

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Abstract

Purpose: To assess the effect of Ahmed glaucoma valve implants on corneal endothelial cell density (ECD) in children with uveitic glaucoma.
Design: Cross-sectional study.
Methods:
Setting: Institutional
Patient Population: 80 eyes from 42 patients diagnosed with uveitis before the age of 16. Twenty-eight eyes had an Ahmed glaucoma valve implant due to secondary glaucoma. Fifty-two eyes without an implant served as controls.
Intervention or Observation Procedure(s): Corneal ECD was examined cross-sectionally using a non-contact specular microscope. Univariate and multivariate generalized estimating equations analyses with correction for paired eyes were performed.
Main Outcome Measure(s): Correlation of ECD with the presence of an Ahmed glaucoma valve implant and with the time following implantation.
Results: ECD was significantly lower in the Ahmed glaucoma valve group than in controls (2359 and 3088 cells/mm2, respectively; $P < 0.001$) following an average of 3.5 years after Ahmed glaucoma valve implantation. Presence of an Ahmed glaucoma valve implant, previous intraocular surgery, age, duration of uveitis and history of corneal touch by the implant tube were all significantly associated with decreased ECD. Following a multivariate analysis, presence of an Ahmed glaucoma valve implant ($B = -340$; adjusted $P < 0.011$) and older age ($B = -58$; adjusted $P = 0.005$) remained independently associated with decreased ECD. Within the implant group, the age-adjusted time interval following Ahmed glaucoma valve implantation was highly correlated with decreased ECD ($B = -558$, $P < 0.001$).
Conclusions: Ahmed glaucoma valve implants in children with uveitic glaucoma are independently associated with decreased ECD, and this effect is associated with the time interval following Ahmed glaucoma valve implantation.

Introduction

Uveitis is an important cause of ocular morbidity in children and accounts for 5-20% of blindness cases in the United States and Europe.[1-4] Glaucoma can arise secondary to uveitis or the corticosteroids used to treat uveitis. Uveitic glaucoma is a common complication that occurs within five years among 12-46% of children with all types of uveitis.[5-7] Legal blindness can be attributed to secondary glaucoma in 15% of children with all types of uveitis, and this prevalence rises to 50% among children with uveitis associated with juvenile idiopathic arthritis (JIA).[7;8]

Children with uveitic glaucoma carry a high risk for trabeculectomy failure. The relatively young age, ocular inflammation and frequent need for cataract surgery among these patients all predispose the patient to excessive fibrosis, which is associated with a less favorable outcome following trabeculectomy.[9] Glaucoma implant valve devices such as an Ahmed glaucoma valve or Baerveldt implant have been proven effective for lowering intraocular pressure in refractory glaucoma.[10-13] However, their long-term effects on the corneal endothelium in inflammatory glaucoma are unknown.

Corneal endothelial cells do not proliferate in humans. Endothelial cell density (ECD) is highest after birth (with a mean cell density of 4000-6000 cells/mm^2), after which it declines rapidly in the first two years of life.[14;15] Known factors for endothelial cell damage include cataract extraction, glaucoma surgery and corneal transplantation.[15-23] Among the available topical drugs, only dorzalomide (a carbonic anhydrase inhibitor) seems to have a possible negative effect on the corneal endothelium.[15]

Two recent studies prospectively evaluated the effect of Ahmed glaucoma valve implants on ECD following one and two years of follow-up and suggested a progressive corneal endothelial cell loss in the eyes that received an implant.[24;25] However, neither of these studies included children, and only a portion of the study populations had glaucoma secondary to uveitis. Therefore, the purpose of the present study was to investigate the effect of an Ahmed glaucoma valve implant on corneal endothelial cells in children with glaucoma secondary to uveitis.

Materials and Methods

In this cross-sectional observational case study, we included all patients who were diagnosed with uveitis before the age of sixteen years and who presented with glaucoma secondary to uveitis and received an Ahmed glaucoma valve implant at the Depart-

ment of Ophthalmology at the University Medical Center, Utrecht, The Netherlands, from March 2005 through December 2011. Children with uveitis who were diagnosed before the age of sixteen and who did not receive an Ahmed glaucoma valve implant served as the control group. The unaffected eyes of unilateral uveitis patients and eyes with phthisis bulbi secondary to longstanding uveitis were excluded. This study was approved by our University's Institutional Review Board and was performed in accordance with the 1990 Declaration of Helsinki and subsequent amendments. Informed consent for the evaluation of the endothelial cell counts and for using their data in this study was obtained from the patients' parents and from children who were older than 12 years of age.

Corneal endothelial cells were examined using a non-contact specular microscope (Topcon Specular Microscope model SP-2000P) by experienced physicians. For each patient, corneal endothelial cell density was assessed once in the central region. A diagnosis of uveitis was based on the criteria of the Standardization of Uveitis Nomenclature (SUN) Working Group.[26] A diagnosis of JIA was made in accordance with the criteria established by the International League against Rheumatism (ILAR).[27;28] Each preliminary presumptive diagnosis of JIA was confirmed by a pediatric rheumatologist. Diagnoses of underlying systemic diseases associated with uveitis were established in accordance with current diagnostic criteria.

The following clinical data were collected from each patient's medical records: gender, date of the onset of uveitis, anatomical type and etiology of uveitis, previous cataract extraction and/or trabeculectomy, topical administration of carbonic anhydrase inhibitors (due to their potential effects on endothelial cell function)[15], pre-operative administration and application duration of wound healing modulators such as 5-fluorouracil and mitomycin C, date and eventual complications of the first Ahmed glaucoma valve implantation such as implant failure or tube-related complications. Previous cataract extraction and/or trabeculectomy were considered to be a previous intraocular surgery.

All surgeries were performed by specialized physicians under general anesthesia. The Ahmed glaucoma valve devices [model S-2 (surface area of 184 mm^2) or model FP8 (surface area of 96 mm^2), New World Medical Inc., Rancho Cucamonga, California] were implanted using a standard technique, and each valve was placed in the superior temporal or superior nasal area.[24] The indication for an FP8 implant was a previous period of hypotony. Each patient was pre-treated with systemic corticosteroids (1 mg/kg) beginning two days prior to surgery and received periocular bethamethasone during surgery. Postoperatively, the systemic corticosteroids were slowly tapered off

based on the activity of the intraocular inflammation.

Statistical analysis was performed "by eye" using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL). Generalized estimating equations with statistical correction to test for correlations between paired eyes were used for univariate and multivariate analyses. Correlations between variables with correction for paired eyes were also analyzed using generalized estimating equations. Differences with $P \leq 0.05$ were considered to be statistically significant. Variables that were found to be statistically significant in the univariate analysis were subsequently included in the multivariate analysis.

Results

Eighty eyes from forty-two patients were included in this study. The characteristics of the study subjects are summarized in Table 1. The Ahmed glaucoma valve group contained 28 eyes from 21 patients with an S2 implant (n = 26 eyes) or an FP8 implant (n = 2 eyes). Seven and 14 patients had bilateral and unilateral Ahmed glaucoma valve implants, respectively. The control group consisted of fifty-two eyes without an Ahmed glaucoma valve and included eyes with bilateral uveitis (n = 36), unilateral uveitis (n = 3) and the unaffected eyes of patients with a unilateral Ahmed glaucoma valve device (n = 13; one unaffected eye of a patient with a unilateral Ahmed glaucoma valve had phthisis and was therefore excluded from the analysis) (Table 1). Both the mean age and the average number of previous intraocular surgeries in the Ahmed glaucoma valve group were significantly higher than the control group (Table 1). There were no statistical differences between the two groups with respect to the other demographic characteristics (Table 1). The only subsequent intraocular surgeries in the eyes in the Ahmed glaucoma valve group were revisions of the implants in six eyes for the following reasons: tube revision due to corneal touch (n = 2), failure of the implant (n = 2), hypotonia (n = 1) and retraction of the tube (n = 1); the mean time until revision was 2.4 years.

The mean ±SD post-implant (an average of 3.5 years following implantation) ECD count in the Ahmed glaucoma valve group was 2359±831 cells/mm2 (range: 864-3810 cells/mm2). In contrast, the ECD count in the control group was 3088±442 cells/mm2 (range: 2085-4195 cells/mm2), which was significantly higher than in the Ahmed glaucoma valve group ($P < 0.001$) (Figure 1).

A univariate analysis revealed that a lower ECD was significantly associated with the following factors: the presence of an Ahmed glaucoma valve implant, previous intra-

Table 1. Baseline characteristics of the eyes of children with uveitis with and without Ahmed glaucoma valve implant.

Characteristics	Ahmed group N of eyes = 28	Control group N of eyes = 52	*P*-value[a]
Number of patients (N = 42)	21	34[b]	NA
Mean±SD age, in years	16.7 ± 5.0	12.7 ± 4.6	***0.011***
Gender			0.220
Male	11 (39%)	22 (42%)	
Female	17 (61%)	30 (58%)	
Anatomic type			
Anterior	24 (86%)	38 (73%)	
Intermediate	3 (11%)	11 (21%)	0.169
Posterior	0 (0%)	1 (2%)	
Panuveitis	1 (4%)	2 (4%)	
Etiology			
JIA	25 (89%)	40 (77%)	
Sarcoidosis	1 (4%)	3 (6%)	0.292
Toxocara	0 (NA)	1 (2%)	
Unknown	2 (7%)	8 (16%)	
Mean±SD age at uveitis diagnosis, in years	4.7 ± 2.1	5.3 ± 2.3	0.882
Mean±SD duration of uveitis in years	12.0 ± 5.4	7.4 ± 4.8	0.888
Previous intraocular surgery[c]			
None	0 (NA)	26 (50%)	***<0.001***
Cataract extraction[d]	26 (93%)	21 (23%)	
Trabeculectomy with wound healing modulators	14 (50%)	14 (27%)	
Administration of topical carbonic anhydrase inhibitor	10 (36%)	23 (44%)	0.474
Mean±SD age at implantation of Ahmed glaucoma valve, in years	13.2 ± 4.4	NA	NA
Mean±SD time following Ahmed glaucoma valve implantation, in years	3.5 ± 2.2	NA	NA

[a] Univariate Generalized estimating equations analysis with correction for paired eyes; Total number of paired eyes in the analysis is 38.

[b] Including 13 patients with unilateral Ahmed glaucoma valve implant, whose unaffected eyes were included in the control group. One unaffected eye of a patient with a unilateral Ahmed glaucoma valve had phthisis and was therefore excluded from the analysis.

[c] 12 eyes with an Ahmed glaucoma valve and 9 eyes without an Ahmed glaucoma valve underwent cataract extraction and trabeculectomy;

[d] Lens status in the Ahmed glaucoma valve group and the control groups: phakic (n=2 and 31, respectively), pseudophakic (n=21 and 20, respectively) and aphakic (n=5 and 1, respectively);

JIA = Juvenile idiopathic arthritis, NA = not applicable

For some variables the total percentages exceed hundred due to rounding.

ocular surgery, age, duration of uveitis and corneal touch (Table 2). In contrast, revision of the Ahmed glaucoma valve implant, perioperative application of wound-healing modulators and the administration of topical carbonic anhydrase inhibitors were not associated with a lower ECD count (Table 2).

The following variables were subsequently incorporated into a multivariate analysis: the presence of an Ahmed glaucoma valve implant, previous intraocular surgery, corneal touch and age. Both the presence of an Ahmed glaucoma valve implant (B = -340, adjusted P = 0.011) and older age (B = -58, adjusted P = 0.005) remained independent factors that were significantly associated with a lower ECD count (Table 2).

Patient age and the duration of uveitis had a strong bivariate correlation (r = 0.887, P < 0.001) and could therefore not be included together in the multivariate model. Therefore, an alternate multivariate model was created using the duration of uveitis as a factor in place of patient age. In this model, the presence of an Ahmed glaucoma valve implant remained significant (P = 0.011). The influence of a longer duration of uveitis on ECD was less strong and less significant (not shown) than the effect of older age in the initial multivariate model (Table 2). Figure 2 shows the differences in the relationship of ECD to patient age in eyes with and without an Ahmed glaucoma valve implant. The eyes with an Ahmed glaucoma valve implant have a steeper decline in ECD

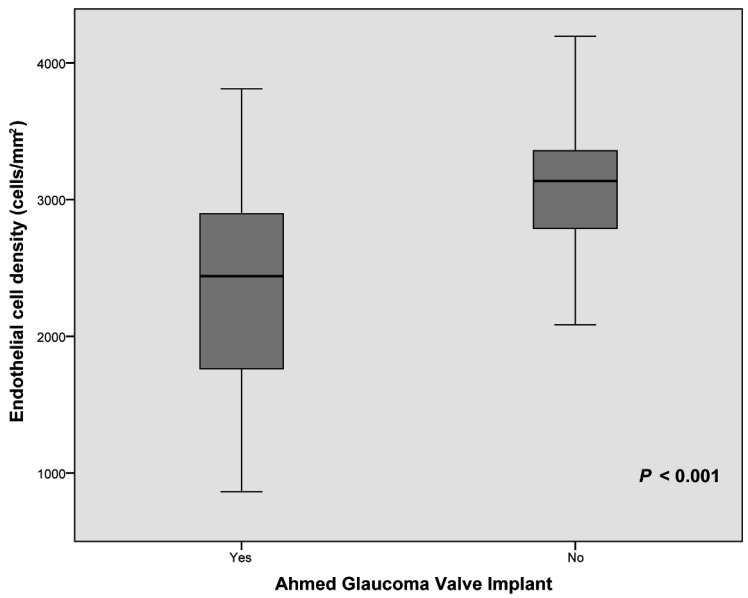

Figure 1. Mean corneal endothelial cell density in the eyes of children with an Ahmed glaucoma valve implant due to glaucoma secondary to uveitis and children with uveitis but without an Ahmed glaucoma valve implant (generalized estimating equations analysis with correction for paired eyes).

Table 2. Results of the univariate and multivariate analysis for factors associated with decreased endothelial cell density in the eyes of children with uveitis.

	Univariate			Multivariate[a]		
Variable	B	95% CI	Crude ***P***-value	B	95% CI	Adjusted ***P***-value
Presence of an Ahmed glaucoma valve implant	-619	-939, -300	***< 0.001***	-340	-603, -77	***0.011***
Age	-77	-121, -33	***0.001***	-58	-99, -18	***0.005***
Previous intraocular surgery[b]	-480	-784, -76	***0.002***	-140	-329, 49	0.147
Corneal touch	-1442	-2640; -243	***0.018***	-1026	-2096, 45	0.061
Duration of uveitis[c]	-57	-94, -20	***0.003***	Not applicable[c]		
Administration of wound-healing modulators	-241	-496, 12	0.062	Not applicable		
Revision of Ahmed glaucoma valve implant	-245	-943, 451	0.490	Not applicable		
Administration of carbonic anhydrase inhibitors	-37	-286, -212	0.771	Not applicable		

B = regression coefficient; 95% CI = 95% Confidence interval

[a] Multivariate generalized estimating equations analysis with correction for paired eyes;

[b] Previous trabeculectomy and cataract extraction added as separate variables added in the multivariate model showed no statistically significant independent effect (Trabeculectomy P = 0.717; cataract extraction P = 0.399), while presence of Ahmed glaucoma valve implant (p = 0.009) kept the significance of its effect;

[c] Age and duration of uveitis had strong bivariate correlation (r = 0.887, P < 0.001) and could therefore not be included together in the multivariate model. Therefore an alternative multivariate model has been built with duration of uveitis as a factor instead of age. In this model, the presence of Ahmed glaucoma valve implant kept the significance of its effect (P = 0.011).

compared to control eyes but have a less significant association with ECD (Figure 2). Within the Ahmed glaucoma valve implant group, the time interval following the Ahmed glaucoma valve implantation was highly correlated with lower ECD counts. Figure 3 shows this relationship and provides a crude regression coefficient and P-value. When adjusted for the effect of age ($r = 0.481$; $p = 0.010$), the time interval following the Ahmed glaucoma valve implantation remained significantly associated with lower ECD ($B = -558$, adjusted $P < 0.001$).

Discussion

The results of this study show that previous Ahmed glaucoma valve implantation is associated with a lower corneal endothelial cell count in children with secondary glaucoma due to uveitis. A functioning endothelium is essential for corneal integrity, and transparency and endothelial cell loss during childhood can lead to corneal edema and ultimately to visual loss.[15;29] A higher rate of endothelial cell loss at a relatively young age can have negative long-term consequences for vision. However, an Ahmed glaucoma valve implant can be effective for managing secondary glaucoma, the major cause of visual loss in childhood uveitis.[7;8;10-13] Therefore, both the risks and benefits of performing an Ahmed glaucoma valve implantation should be weighed carefully in these patients; to date, no corneal decompensation has occurred in our population. However, the lowest ECD in our study (864 cells/mm^2 six years following Ahmed glaucoma valve implantation) approaches the critical limit of ECD values at which corneal decompensation can occur.

The time interval following the Ahmed glaucoma valve implantation adjusted for normal aging effects was found to be strongly associated with lower ECD, which suggests a higher rate of endothelial cell loss than occurs in individuals who have not undergone this surgery. This observation is consistent with previous studies in adults that have shown that corneal ECD decreased incrementally at 12 and 24 months following Ahmed glaucoma valve implantation.[24;25] Although the largest decrease in ECD in these studies occurred in the region closest to the tube, the central area also showed a significant decrease in ECD starting six months following the Ahmed glaucoma valve implantation.[24;25] Our study describes the effect of the time interval following Ahmed glaucoma valve implantation on central ECD. Our decision to measure central ECD was based on the desire to measure the most clinically relevant ECD in the visual axis and to perform comparisons with eyes that contain no tube. Furthermore, performing

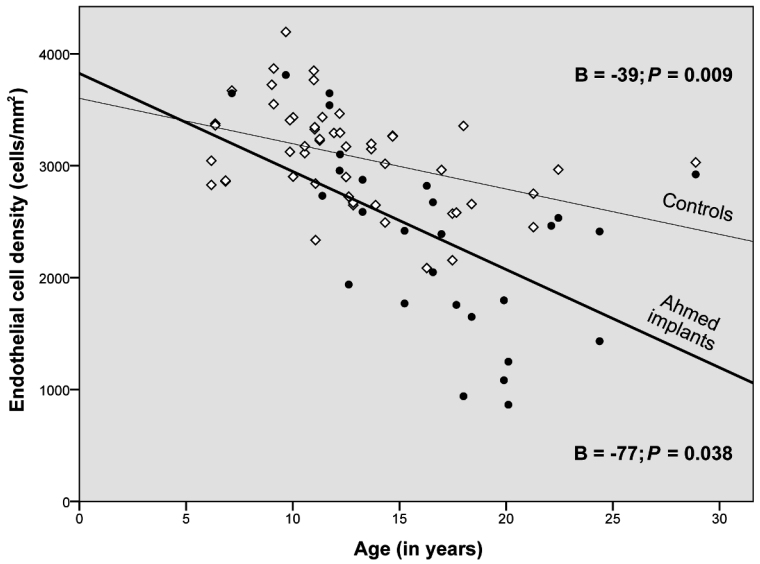

Figure 2. Corneal endothelial cell density with respect to patient age in the eyes of children eyes with or without an Ahmed glaucoma valve implant (adjusted for paired eyes in the analysis). The solid circles represent the endothelial cell density values of individual eyes with an Ahmed glaucoma valve implant and the open diamonds represent individual control eyes. B (the regression coefficient) is given for the Ahmed glaucoma valve implant group and the control group.

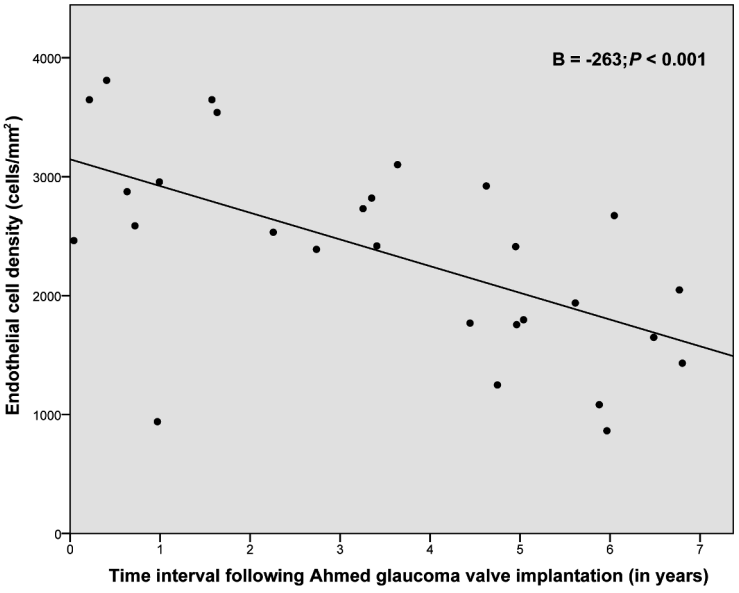

Figure 3. Corneal endothelial cell density with respect to the time interval following Ahmed glaucoma valve implantation in the eyes of children with an Ahmed glaucoma valve implant due to glaucoma secondary to uveitis (adjusted for paired eyes in the analysis, but not for other factors). B (the regression coefficient is given).

a uniform central measurement both increases reproducibility and is easier to perform in a young child.

Age is a factor that is generally known to affect ECD. ECD is highest at birth and declines rapidly in the first two years of life.[14;15] From then, the decline continues, but at a slower rate of 0.30-0.71% per year, leading to a mean cell density of 2800 cells/mm^2 in adults.[14] Uveitis could facilitate this progressive ECD loss. However, in our analysis, the effect of a longer duration of uveitis seemed to be minor relative to the effects of other factors. The children with an Ahmed glaucoma valve implant were significantly older than the children with no implant. This difference can be attributed to the fact that Ahmed glaucoma valve implants are generally being used relatively late in the course of the disease, after a trabeculectomy has failed. Ahmed glaucoma valve implants have been used at our center since 2005. In our study, the effect of an Ahmed glaucoma valve implant on endothelial cell count in our study was adjusted for age in our multivariate models. Our results show that an Ahmed glaucoma valve implant has a negative effect on ECD independent of the effects of normal aging. However, because this study was cross-sectional in design, it is not possible to draw conclusions regarding the precise correlation in time between endothelial cell loss and the time interval following Ahmed glaucoma valve implantation.

Trauma to the corneal endothelium which can occur during intraocular surgery can reduce cell density, increase cell size and disrupt the normal morphological pattern.[27] Mean corneal endothelial cell losses following glaucoma filtering surgery are reported to range from 0.2 to 14.9%, and after cataract extraction with phacoemulsification, losses range from 1.2 to 14.1% one year following surgery.[22-26;28;29] However, in our study, previous intraocular surgery did not retain its significance in the multivariate analysis when adjusted for other factors. Moreover, analyses with trabeculectomy and cataract extraction as independent variables revealed no statistically significant association with lower ECD. However, our study focused exclusively on cell density and did not include cell shapes and patterns that can also serve as indicators of endothelial damage.[21] Therefore, it will be interesting to study these patterns in children with and without an Ahmed glaucoma valve implant.

The main limitation of this study is its cross-sectional design, which used a single ECD measurement and lacked pre-operative ECD measurements. However, this is the first study of the effect of Ahmed glaucoma valve implants in children with uveitis to suggest that the implant has a negative effect on ECD and can therefore serve as a starting point for further prospective research. A longitudinal study using ECD measurements obtained pre-operatively and at several postoperative time points may provide more

accurate information regarding the longitudinal relationship between time following the implant and ECD loss and may help clinicians predict the long-term risks to these patients. Our observations are therefore likely to be valuable to ophthalmologists who treat uveitic glaucoma in children.

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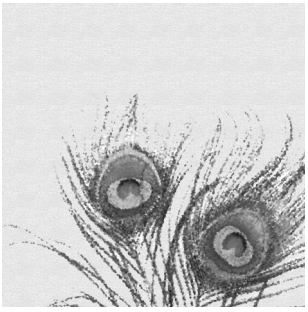

Chapter 12

Prospective study on ocular complications in children within 1 year after hematopoietic stem cell transplantation (HSCT)

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Abstract

Purpose: To prospectively study development of ocular complications in children within 1 year after hematopoietic stem cell transplantation (HSCT) and specifically to investigate the risk of development of viral uveitis in immunocompromised children after HSCT.

Design: Prospective cohort study.

Participants: 49 consecutive patients who underwent a HSCT in the UMC Utrecht, Netherlands in 2009-2010.

Methods of testing: The patients underwent systematic ophthalmologic evaluations before HSCT, before leaving the HSCT unit post-HSCT and after 3, 6 and 12 months post-HSCT. Additional examinations were performed during systemic viral reactivations.

Main outcome measures: Development of ocular complications including among others uveitis, hemorrhagic complications, optic disc edema and dry eye syndrome (DES).

Results: Thirteen patients (27%) developed an ocular complication post-HSCT. These included: DES (n = 7; 14%); (sub)retinal hemorrhage (n = 6; 12%); optic disc edema (n = 3; 6%), chorioretinal lesions (n = 2; 4%), vitritis (n = 1; 2%) and increased intraocular pressure (n = 1; 2%). Median time to development of DES was 5 months post-HSCT, while all other ocular complications were detected within the first 3 months after SCT. In most cases the symptoms were mild and self limiting, although a potential danger of a vision-threatening condition does exist. Children with a malignant indication for HSCT had higher risk for development of ocular complications compared to children with non-malignant diseases.

Conclusions: Ocular complications in pediatric HSCT-patients are common, although mostly mild. The risk of viral uveitis development during systemic viral reactivations is low. However, potential risk of vision-threatening complications in this population can not be ruled out. We recommend performing ophthalmologic examination pre-HSCT, in case of systemic infectious reactivations and apparently when visual symptoms are recorded. Groups of patients with highest risk of ocular complications, namely patients with malignancies and children of pre-amblyopic age, deserve higher awareness.

Introduction

Allogenic hematopoietic stem cell transplantation (HSCT) has gradually gained a broad application in treatment of malignant (mainly leukemias and lymphomas) as well as non-malignant diseases (immunedeficiencies, hemoglobinopathies and metabolic diseases) in children. Success rate and survival after this treatment are still increasing. Nevertheless various side effects may affect different organs and may result in impaired "quality of life" after this potentially life-saving treatment.

The eye can be affected in both early and late stages after HSCT. The ocular damage can affect all parts of the eye and it might represent a result of the immunosuppression, conditioning regime (chemotherapy and/or irradiation) and/or underlying disease.[1,2] This applies to adult as well as the pediatric population where development of ocular surface disease, cataract and posterior segment complications after an HSCT have been described in retrospective and cross-sectional studies.[3-8]

Opportunistic infections due to the immunosuppressed status in the first months after HSCT play a major role in the morbidity and the mortality of these patients. These infections may affect the eye severely; however no systematic prospective studies have been performed to investigate the exact risk of ocular pathology in the pediatric HSCT population.[1;3;9-17] Due to the improving clinical control of these infections and lower mortality, it is essential to have insights in the risk of ocular involvement following HSCT. In addition, it is crucial to develop adequate screening guidelines for this vulnerable population, as young and severely ill children are not aware of their ocular problems.

This prospective study describes ocular findings in children before and during the first year after an allogenic HSCT. We specifically focus on the eventual ocular involvement during systemic viral reactivations. Furthermore, we report on the moment of development of ocular complications within the first year post-HSCT in order to investigate whether ophthalmologic screening is warranted for pediatric HSCT population

Patients and Methods

This prospective study includes all consecutive patients who had undergone a HSCT at a pediatric Hematology and Immunology Department of the Wilhelmina Children's Hospital/University Medical Center Utrecht, the Netherlands in 2009 and 2010. Transplantation details, conditioning regimens, supportive care, graft-versus-host disease

(GVHD) prophylaxis and infection monitoring within the "pediatric blood and marrow transplantation program" of the UMC Utrecht (Wilhelmina Children's Hospital) have been extensively described and published previously.[18;19] The study was performed with an institutional ethical committee approval.

Briefly, viral pre-HSCT prophylaxis was given only in cases of positive serology for herpes simplex virus (HSV) (with intravenous acyclovir during neutropenia and orally with valacyclovir after neutropenia until number of CD4+/ul > 200/uL, 500mg/m^2). Prophylaxis for other viruses was not given. Total IgG levels were checked every 2 weeks; intravenous immunoglobulin was given only to those patients with an IgG level <4 g/L. For viral monitoring, plasma samples were tested weekly for Epstein-Barr virus (EBV), cytomegalovirus (CMV), human herpes 6 virus (HHV6), and adenovirus DNA positivity by quantitative real-time polymerase chain reaction (PCR). CMV, HHV-6, Adenovirus and EBV were preemptively treated according to local guidelines depending on cell source used, time after transplant and immune reconstitution [18]. The skin was checked for VZV and HSV reactivation during physical examinations.

Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine (aiming for a trough level of 100-250 µg/L, based on national protocol guidelines), supplemented with methylprednisolone in patients receiving a cord blood (CB) transplant, or short course of methotrexate in patients receiving an unrelated bone marrow (BM) or peripheral blood stem cell (PBSC) transplant. In patients receiving an unrelated donor graft (CB, BM, or PBSC), antithymocyte globulin (ATG) serotherapy was added to the conditioning regimen. [18] Patients were regularly evaluated by the HSCT-unit staff for presence and severity of acute or chronic GVHD.

Ophthalmologic examinations were performed by pediatric ophthalmologists and all children were additionally examined by an orthoptist. All included children had an ophthalmologic evaluation before HSCT. After HSCT the patients were regularly examined before leaving the HSCT-unit (median time of 3 weeks after HSCT), and later on at 3, 6 and 12 months after the SCT. Additional ophthalmologic examinations were performed in cases of a viral reactivation of CMV, VZV, HSV, EBV, HHV-6 and adenovirus and/or if ocular complains were present. The examinations were adapted to the age and co-operability of every child. Standard ophthalmologic examination included visual acuity measurement, slit lamp examination (including fluorescein staining of the cornea) and fundoscopy with dilated pupil. Examination at the HSCT-unit contained in all cases a funduscopy with dilated pupil and in case of ocular complaints a visual acuity measurement and hand slit-lamp examinations were also performed. Additional tests, such as visual field examinations, were based on clinical findings. In

case of a second HSCT in the same patient the schedule of ophthalmologic examinations was repeated.
Dry eye syndrome (DES) was defined as presence of corneal epithelial staining in combination with a reduced tear film by slit-lamp examination.
For statistical data analysis SPSS version 15.0.1 (SPSS Inc, Chicago, Illinois, USA) was used. Kolmogorov-Smirnov test was used to analyze the normality of data distribution. Mann-Whitney U-test was used for non-parametric analysis of differences between the groups. Cox proportional hazard model was used to analyze associations between variables and outcome. Duration of follow-up was the time to last ophthalmologic examination for survivors or the time until death. To analyze predictors for development of DES and hemorrhages we considered the following variables: recipient associated: age at HSCT, gender, malignancy as indication for HSCT; transplantation associated: source of stem cells, human leukocyte antigen (HLA) disparity and the basis of the conditioning regimen (chemotherapy or total body irradiation). A stepwise forward regression was performed using a threshold of 0.05. All tests are 2-sided. P-values of < 0.05 were considered significant.

Results

A total of 49 patients were included in this study. Forty-eight patients received an allogenic and one patient an autologous HSCT. In two patients an allogenic HSCT was preceded by an autologous HSCT. Demographics, indications for HSCT and main patient characteristics are presented in Table 1. The median age of patients at the time of the HSCT was 6.6 years. Thirty-two patients (65%) completed 1 year of the ophthalmologic follow-up post HSCT (Table 1). Fourteen patients (29%) died within the first year post HSCT and three patients (6%) were lost to follow-up (Table 1).

Ophthalmologic findings pre-HSCT

Pre-HSCT ophthalmologic evaluation revealed abnormal findings in 4/49 patients (8%) previously un-transplanted patients. These findings included mild dry eye syndrome (DES, n = 2), retinal hemorrhages (n = 1, also DES was present simultaneously in this patient), optic disc edema (n = 1) and chorioretinal scars (n = 1; Table 2). Bilateral mild swelling of the optic disc was detected pre-HSCT in one patient with acute lymphoblastic leukemia (ALL) without any signs of intraocular inflammation. The findings could not be clarified by a relapse of theunderlying disease and/or raised

Table 1. General characteristics of 49 children patients with hematopoietic stem cell transplantation (HSCT).

Characteristics		Total N=49
General patient characteristics		
Gender (n;%)	Female Male	19 (39) 30 (61)
Age (years) at HSCT (median; range)		6.6 (0.2-22.7)
Indication (n;%)	Malignant disorder Non-malignant disorder	28 (57) 21 (43)
Diagnosis: (n;%)		
Acute Lymphoblastic Leukemia		12 (25)
Acute Myelogenous Leukemia		5 (10)
Fanconi anemia		4 (8)
Juvenile Myelomonocytic Leukemia		4 (8)
Non-Hodgkin lymphoma		3 (6)
Metachromatic leucodystrophy		3 (6)
Myelodysplastic syndrome		2 (4)
Mucopolisaccharidosis I Hurler		2 (4)
Hemophagocytic lymphohistiocytosis		2 (4)
Common variable immunodeficiency		2 (4)
Cronic granulomatous disease		2 (4)
Miscellaneous*		7 (14)
HLA disparity (n;%)	Matched Mismatched	30 (61) 19 (39)
Source stem cells (n;%)	Bone marrow / Peripheral blood Cord blood	31 (64) 18 (36)
Conditioning	Total body irradiation-based Chemotherapy-based	38 (78) 11 (22)
Ophthalmologic follow-up		
Follow-up months (median; range)		6 (0.5-12)
Patients with completed 1 year ophthalmologic follow-up post HSCT (n;%)		32 (65)
Patients with completed 6 months ophthalmologic follow-up post HSCT (n;%)		38 (78)
Patients with completed 3 months ophthalmologic follow-up post HSCT (n;%)		45 (92)
Patients died within 1 year post HSCT (n;%)		14 (29)
Patients lost to follow-up within 1 year post HSCT (n;%)		3 (6)

* Miscellaneous included Kostmann syndrome, Omenn syndrome, osteopetrosis, CD40 ligand deficiency, Hodgkin lymphoma, Burkitt lymphoma, alpha-thalassemia and mitochondrial neurogastrointestinal encephalopathy syndrome (MNGIE).

intracranial pressure or toxic etiology. Peripheral chorioretinal punched-out lesions were detected pre-HSCT in a patient with x-linked chronic granulomatous disease (CGD) (Figure 1).
The lesions appeared inactive and were clinically consistent with chorioretinal lesions that have been previously described for patients with this disorder.[20-23]

Ophthalmologic findings post-HSCT

In total, new abnormal eye conditions were detected in 13 patients (27%) within 1 year post-HSCT. Table 2 shows an overview of detected abnormalities, the moment of their detection and underlying diseases of the affected patients. Seven patients (14%) developed DES and remaining patients had retinal hemorrhages (n = 6; 12%), optic disc edema (n = 3; 6%), chorioretinal lesions (n=2; 4%), vitritis (n = 1; 2%) and increased intra-ocular pressure (IOP) (n = 1; 2%) (Table 2). Figure 2 shows cumulative incidence curve of ocular complications.

Abnormal findings in early post transplantation period (within first 3 months post-HSCT) Systemic viral reactivations occurred in 20 patients (CMV n = 9; VZV n = 6; HHV-6 n = 4, adenovirus n = 3 en EBV n = 2), in 4 patients more than one different viral reactivations occurred. Significantly more patients with ALL had viral reactivations compared with patients with other diagnoses. (83% vs. 27%; p = 0.001). All patients underwent ophthalmologic examination during their viral reactivation, but no new ocular abnormalities were observed in that time.
However, new asymptomatic chorioretinal scars were observed after 3 and 5 weeks post-HSCT in two ALL patients after having systemic CMV (treated with foscavir) and VZV (treated with valacyclovir) reactivations in a very early post-transplantation period (Table 2). Round multiple central and peripheral punched-out lesions were located bilaterally in the young patient with previous VZV reactivation (Figure 3). The patient with previous CMV reactivation developed multiple round punched-out lesions located only peripherally. No active intraocular inflammation was observed; therefore no additional diagnostic procedures were performed.
Retinal hemorrhages were detected in 6 patients (12%) (Table 2), in all located intra-retinally and in one patient also a sub retinal hemorrhage was present (Figure 4). Hemorrhagic complications typically developed in the early post-transplant period (Table 2). The hemorrhages did not threaten the vision due to their peripheral localization and were in all cases associated with a deep thrombocytopenia with the lowest thrombocyte count in 2 weeks before detecting the hemorrhage ranging between 4 and 24 x

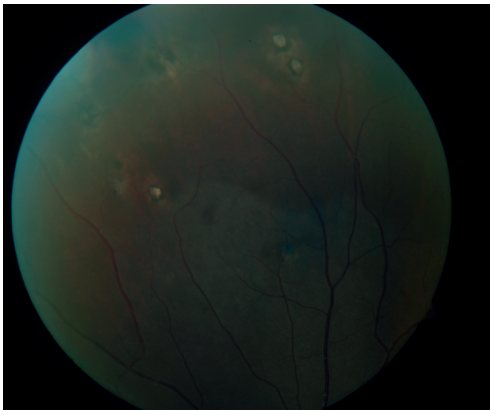

Figure 1. Inactive chorioretinal lesions located (mid)peripherally in both eyes of a patient with chronic granulomatous disease (CGD), detected at a pre-HSCT examination.

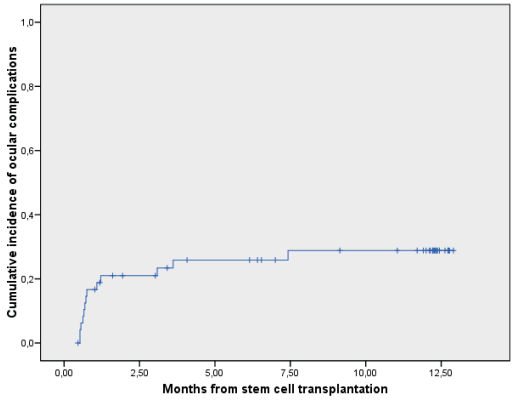

Figure 2. Cumulative incidence of ocular complications in children within 1 year after a stem cell transplantation (HSCT).

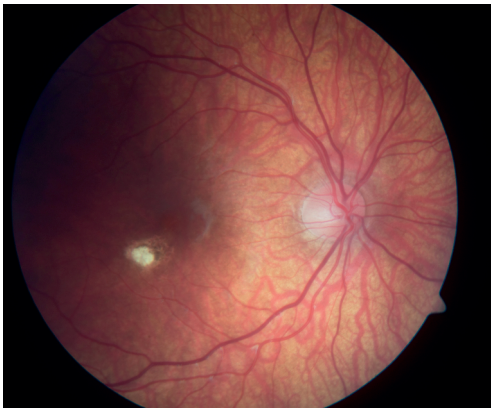

Figure 3. Central chorioretinal lesion detected de novo post-HSCT in a patient with ALL and a history of VZV reactivation, treated with valaciclovir. Several similar inactive lesions were also located peripherally in both eyes.

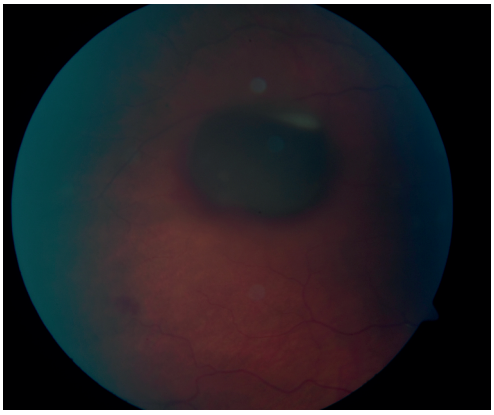

Figure 4. Subretinal haemorrhage located peripherally in a patient with severe thrombocytopenia post-HSCT.

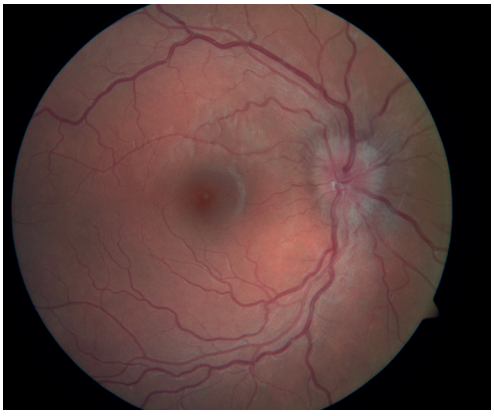

Figure 5. Optic disc edema in a patient with ALL during systemic administration of cyclosporine. Edema was located bilaterally.

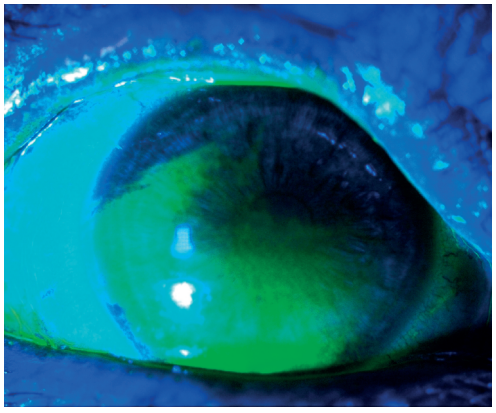

Figure 6. Fluoresceine staining of the cornea in a patient with a severe dry eye syndrome (DES) post-HSCT in a setting of graft-versus-host disease (GVHD).

Table 2. Abnormal eye conditions detected in pediatric patients before- and within 1 year after hematopoietic stem cell transplantation (HSCT).

Abnormal eye conditions	Pre-HSCT N=49	Post-HSCT[a] N=49[b]	Time post-HSCT (median; range)	HSCT indications (n)
Total number of patients with ocular abnormalities (n;%)[c]	4 (8)	13 (27)		
Dry eye syndrome (DES)	2 (4)	7 (14)	5 months (3 weeks - 12 months)	ALL(1); AML(2); NHL(2); MDS(1); CGD(1)
(Sub)retinal hemorrhage	1 (2)	6 (12)	3 weeks (2 - 5 weeks)	ALL(2), Burkitt-lymphoma(1); MDS(1); NHL(1); CGD(1);
Optic disc edema	1 (2)	3 (6)	3 weeks (3 - 5 weeks)	ALL(2); NHL(1)
Chorioretinal lesions	1 (2)	2 (4)	3 weeks and 5 weeks (NA)	ALL(2)
Vitritis	NA	1 (2)	3 months (NA)	AML
Increased intraocular pressure	NA	1 (2)	3 months (NA)	NHL

[a] New onset ocular findings after HSCT

[b] The percentages are computed regardless loss to follow-up.

[c] Multiple abnormal findings might be present in one patient

NA = not applicable

Diagnoses: ALL = acute lymphoblastic leukemia; AML = acute myelogenous anemia; NHL = non-Hodgkin lymphoma; MDS = myelodysplastic syndrome; CGD = cronic granulomatous disease.

10^9/l. The hemorrhages resolved after improvement of thrombocytopenia. In Cox regression analysis of HSCT-related factors development of hemorrhagic complications was associated only with older age at HSCT (adjusted HR = 1.21; 95% CI 1.04-1.40; p = 0.013).

Optic disc edema was seen in 3 patients (6%) during a post-SCT period (Table 2). One patient with ALL and a mild idiopathic bilateral swelling of the optic disc pre-HSCT developed a significant increase of the edema post-HSCT (Figure 5) during systemic administration of cyclosporine. An extensive diagnostic workup excluded increased intracranial pressure, infection or central nervous system relapse of leukemia. The remaining two cases of the optic disc edema in post-HSCT period were detected within first 5 weeks after the transplantation. In both patients optic disc edema was transient (one had a bilateral and the other a unilateral edema) and the cause(s) remained not identified.

In one patient without concurrent proven reactivation of infection, a mild bilateral vitritis with hyperemic optic discs was seen at 3 months post SCT. The patient died before the next planned ocular examination.

Bilaterally increased intraocular pressure of 25 mmHg was detected in one patient at 3 months post-SCT in combination with bilaterally new onset small excavations of optic discs under administration of oral corticosteroids. The patient died before the next planned ocular examination.

Abnormal findings later in post transplantation period (3-12 months post-HSCT)

New onset of DES was observed in 14% (7/49) of the patients (Table 2). Median time to detection of DES was 5 months and ranged from 3 weeks to 12 months. While DES was in our study a typically later finding, 2 previously un-transplanted patients had signs of DES within 3 months post-HSCT.

In 4 out of 7 patients with DES (57%) the findings were accompanied by characteristic systemic manifestations of acute (n = 2) and/or chronic (n = 3) GVHD in at least 1 other organ. In patients without DES systemic GVHD was diagnosed in 31% of patients, however, this difference was not statistically significant. Patients with DES were significantly older than other patients (median age 13.7 versus 5.5 years; p = 0.003). One patient with systemic GVHD developed severe DES which required intensive therapy including lubricants, steroid drops, cyclosporin ointment, autologous serum drops and bandage contact lenses (Figure 6). This patient developed subsequently herpetic keratitis. All other cases of DES were relatively mild and the symptoms could be well controlled with (temporary) administration of lubricants (n=5) or even without

therapy (n=1). In Cox regression analysis development of new onset DES post-SCT was independently associated with older age at HSCT (adjusted hazard ratio [HR] = 1.22; 95% CI: 1.05-1.42; p = 0.011).
Out of 13 patients with ocular abnormalities occurring in the post-transplant period, 12 (92%) had a malignant indication for HSCT. Within the patients with malignancies 42% (12/28) developed ocular abnormalities compared with 4% (1/21) in patients with non-malignant diseases (relative risk = 10.5; p=0.003).
Four patients had multiple abnormal ocular findings post-HSCT. All of them had signs of DES in combination with other complications. Only 5 of 13 (38%) patients with ocular abnormalities had subjective complaints; four with DES and 1 with optic disc edema.
Five out of 13 patients (38%) with abnormal ophthalmologic findings died within the first year post-HSCT. In patients with no ocular complications the mortality rate within the first year post-HSCT was 25%. This difference was not statistically significant.

Discussion

During this prospective study no signs of active viral, fungal or bacterial infections of the eye were detected, despite the fact that 41% of the patients developed a systemic viral reactivation. However, new onset inactive chorioretinal scars were observed in two patients after recovery from a viral reactivation, but their potential infectious etiology remained unclear. Ocular abnormalities were detected in 27% of pediatric HSCT patients in the studied cohort within 1 year after a HSCT. Most of detected abnormalities were relatively mild and not vision-threatening, including DES and retinal hemorrhages as most frequent findings. Ocular abnormalities were observed significantly more frequently in patients with malignant disease as indication for HSCT.
Opportunistic infections of the eye are infrequent but most deleterious and vision-threatening complications in a post transplantation period in adults.[1;9-11;16] In children, these complications fortunately seem to be very rare which is consistent with our observations.[3;4;7;8;12-14] Low incidence of these vision devastating complications is an assumable result of significant progress which has been made in the prevention of the post transplantation infections in HSCT patients. However, pediatricians and ophthalmologists should be aware of the possibility of developing such severe infections.
New chorioretinal lesions were detected in early post transplant period in two boys with ALL. These lesions were similar to the lesions of a patient with ALL described previously by Ng et al.[8] Both our patients had history of a systemic viral reactivation

in a very early post transplantation period and were treated with antiviral agents. The character of the chorio-retinal lesions was not typical for VZV or CMV retinitis,[1;11] although the possibility of transient local retinitis under administration of antiviral agents cannot be excluded. The hypothesis of choroidal vascular occlusions as a cause of these lesions was suggested,[8] however the history of our patients provides no additional support for this assumption. The history of viral reactivations in the case described by Ng et al is unknown.

Cumulative ocular complication rates in pediatric HSCT population reported in retrospective studies varied between of 33 and 51%.[4;7] These complication rates also included long-term complications as cataracts, while focus of our prospective study was set on type and timing of ophthalmologic abnormalities within the first year post-HSCT. One retrospective study reports 1 year post-transplantation ocular complication rate of 16%, but this study encompasses both bone marrow as well as organ transplantation recipients.[3] A retrospective character of this study could clarify differences in reported complication rates with our study. The complication rate of our study is calculated regardless loss to follow-up, so the reported incidence could be underestimated.

Our most frequent ocular finding post-HSCT consisted of DES with corneal staining which was detected in 14% of the patients. Earlier retrospective and cross-sectional pediatric reports have described the occurrence of DES with corneal staining ranging from 4 to 62%.[3;4;6-8] An outlier of 62% could be caused by a significantly older population (median age 15.6 years) and a longer follow-up (median 7 years) in that particular study.[6] We show the significant association of DES with older age in pediatric/ young adult population after a HSCT, which was noted earlier.[6] This association is supported by previously described continual decline in tear function with age[24] and association of older age with development of systemic GVHD,[25-27] which seems to be the main underlying reason of DES after a HSCT.[7;8;28] Younger patients probably have better thymic function and higher regenerating ability of cells damaged by GVHD. Other factors which are being traditionally associated with development of DES post-HSCT are total body irradiation and chemotherapy.[6;28] We did not find an association of DES development with other factors than age. It has been suggested that many children may already have DES prior to HSCT.[6] We can not confirm this statement as in our prospective study only 4% of previously un-transplanted patients were diagnosed with mild symptom-free DES pre-HSCT.

Hemorrhagic complications in early post transplantation period were second most frequently detected abnormality in our study. Our findings are in line with earlier studies in children and adults which also report hemorrhagic (pre)retinal complications

associated with low platelet count within first 6 months after the transplantation.[1;4] However, the reported frequency of hemorrhagic complications (3,5 and 4%) seem to be underestimated in these studies due to their retrospective nature and asymptomatic and transient character of the hemorrhages.

The frequency of optic disc edema found in our study (6%) is similar to earlier reports in children and adults (3-5%).[1;4;7;11] The exact cause of the optic disc edema is not known but possibility of cyclosporine toxicity was previously suggested.[1;4;11] This etiology was also presumed in 2 of our patients with transient bilateral optic disc edema with an improvement once the dose of cyclosporine was adjusted. However, unilateral disc edema related to the cyclosporine toxicity is not likely.

Cataracts are one of important delayed ocular complications of HSCT repeatedly reported in children in varying prevalence of 6-58% of the patients depending on duration of follow-up and conditioning regimens.[3-5;7;8] Cataracts are however a rare finding within the first year post transplantation.[3-5;7;8] In our study limited to one year follow-up we did not detect any clinically significant lens opacities. It may be of interest to examine the patients included in this cohort in a 5 and 10 years period again.

All but one patient with ocular abnormalities in our study had malignant diseases. The increased risk of ocular complications in children with malignancies post-HSCT was described earlier.[7] An explanation may be the fact that immunosuppressive agents are tapered earlier in an individual with a malignant indication compared to patients with a non-malignant indication. Earlier taper may be associated with some more alloreactivity in the eye and other organs.

Most of the complications detected in our study were mild; however the potential risk of development of vision-threatening complications in this population remains. Asymptomatic character of these potentially severe complications (for instance, central chorioretinal scars) makes their diagnosis challenging as severely ill children undergoing HSCT might not be aware of decreased vision. Complications located in the visual axis in young children, before the age of eight years, can lead to amblyopia and timely recognition is essential for preventing its development. Also the diagnosis of opportunistic ocular infections can be challenging in pediatric population. Although the patients nowadays are being carefully systemically screened and if necessary treated for reactivations of infections, the presence of intraocular infections might not be suspected by pediatrician. In case of ocular infectious complications, the local treatment (eg with intravitreal injections) might be effective in addition to systemic antiviral treatment and contribute to a better visual outcome. This could make ophthalmologic evaluations of these children useful, although no active intraocular infections were

observed in our study. Therefore, it is important in this population to find a balance between overloading of too much screening and missing clinically relevant findings.
Main goal of post transplantation screening examinations within first year after HSCT should be limitation of eventual damage and long-term visual sequels. Since no preventable causes of decreased vision were detected during screening in our series, the question remains whether ophthalmic screening of all children after HSCT is useful. We find a pre-HSCT examination necessary for detecting ocular abnormalities which require prophylactic treatment during the immunosuppression period, for instance, chorioretinal scars of infectious etiology which can reactivate after the transplantation. Although the frequency of these abnormalities is low, the consequences of such a reactivation might be devastating for vision. Pre-SCT screening is also essential for better appreciation of eventual chorioretinal changes after HSCT.
In conclusion, ocular side effects in pediatric HSCT-patients are common, but most of them are mild with no long-term visual sequels. Although a potential risk of development of vision-threatening complications can not be ruled out completely in this vulnerable group of patients. The results of our study do not allow concluding that a more aggressive screening program is useful. However, we recommend performing ophthalmologic examination pre-HSCT, in case of systemic infectious reactivations and apparently when visual symptoms are recorded. Both ophthalmologist and pediatrician should be aware of groups of patients with highest risk and/or greater impact on long-term vision, namely patients with malignancies and children of preamblyopic age.

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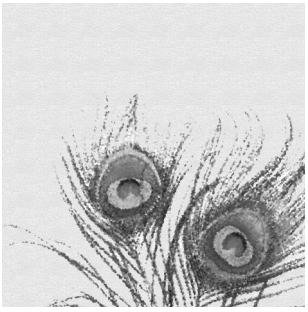


Chapter 13

Summary and conclusions

Summary

Chapter 1 of this thesis provides a general overview of uveitis in childhood. Although uveitis in childhood is a relatively rare condition, it has a significant impact in ocular morbidity and visual disability in children suffering from this disorder. Uveitis in childhood is a potentially blinding disease due to frequently occurring vision-threatening complications in these patients. Its onset is often asymptomatic, but even in advanced cases young children are unaware of their visual disability and usually do not complain. In consequence, the diagnosis of uveitis in a child is regularly delayed and severe complications can be already present at the first ophthalmologic presentation. However, detection of uveitis signs in a child is only a first step in the diagnosis of the cause of uveitis. The subsequent work up should focus on the detection of underlying systemic or infectious causes of uveitis. The main systemic association of uveitis in children is juvenile idiopathic arthritis (JIA), which accounts for at least 30% of uveitis cases in childhood. In the other cases, even after an extensive diagnostic work-up, an idiopathic nature of uveitis is being frequently concluded. Second most common uveitis entity in children is intermediate uveitis, which is almost always idiopathic in pediatric population. The exact pathogenesis of intraocular inflammation in children is not yet clarified, however it is generally accepted that it is an autoimmune disorder in which T cells play the main role. Management of uveitis in children is usually prolonged, complex and challenging. Although locally administered corticosteroids represent the first line treatment, systemic treatment with immunosuppressive drugs is warranted in many cases. Methotrexate is frequently the systemic agent of the first choice, especially in patients with JIA. Complications of childhood uveitis frequently require surgical treatment.

This thesis aimed to gain new insights regarding the course and prognosis of uveitis in childhood (**chapter 3-5;9**), the pathogenesis of JIA-associated uveitis (**chapter 7-8**) and the treatment of uveitis in children (**chapter 6;11**).

Finally we wanted to study prospectively ocular complications in immunocompromised children after hematopoietic stem cell transplantation (HSCT) and specifically to evaluate their risk of uveitis development during systemic viral reactivations. Further, we investigate whether a routine ophthalmologic screening is warranted for pediatric HSCT population (**chapter 12**).

Chapter 2 is devoted to the complex pathogenesis of JIA-associated uveitis. We review the literature and summarize the current knowledge regarding the pathogenesis of

JIA-associated uveitis. We discuss the possible role of immune responses and cytokine involvement, genetic associations and the influence of external triggers in this disease, supported by data obtained from experimental uveitis models.
All of the combined data point to the development of aberrant immune responses involving both the innate and adaptive immune responses. CD4+ T cells are the primary cell type involved in the pathogenesis of JIA-associated uveitis. Specifically, CD4+ T cells serve as both effectors (T helper (Th) 1 and Th17) and regulators of the inflammatory process (T regulatory cells (Tregs)). An imbalance between pro-inflammatory Th1/Th17 cells and anti-inflammatory Tregs is believed to play the crucial role in the inflammation in JIA. In contrast, the role of B cells has been studied less extensively and it is yet unresolved, but these cells may also play a role in the pathogenesis of JIA. B cells and plasma cells (terminally differentiated B cells) are being described as the most abundant cell type infiltrating the eye in JIA-uveitis. However, these findings are primarily based on enucleated eyes, thus representing the end-stage of the disease process, which might be distinct from the earlier active stages of intraocular inflammation in JIA. JIA-associated uveitis is commonly marked by antinuclear antibody positivity, which reflects the production of abnormal self-targeting antibodies in this disorder; however, the nature of the self-antigen remains unknown, and the role of these antibodies in the pathogenesis of the disease is still unclear.
JIA-associated uveitis appears to have a genetic predisposition, particularly with respect to human leukocyte antigen (HLA) class II genes; to date, however, only a protective role of HLA-DR1 has been independently confirmed. JIA-associated uveitis has a relatively low concordance rate among twins, which suggests the involvement of external (non-genetic) factors. Although many external factors, including a variety of infections, have been proposed to increase the risk of developing JIA, none of these factors has been adequately confirmed. The development of chronic and recurrent inflammation in the eyes and joints suggests a similarity between these two organs, although this mechanism and eventual similarities remain unclear.

In **chapter 3-4** we analyze the role of baseline factors in long-term development of ocular complications (**chapter 3**) and in visual outcomes (**chapter 4**) in JIA-associated uveitis. We performed retrospective analysis of data from 117 affected eyes of 65 patients with JIA-associated uveitis with median follow-up of 7.6 years (minimally 1 year). The prognostic roles of gender and age of onset and atypical manifestation of JIA with initial uveitis before arthritis have been specifically analyzed. In both chapters we document the association of male gender with poor prognosis in JIA-associated uveitis. We

found significantly more complication in boys, compared to girls: at 5 years of follow-up boys suffered more frequently from cystoid macular edema (50% vs 4%) and papillitis (31% vs 2%), and needed cataract surgery more frequently than girls (59% vs 32%). Uveitis was diagnosed before arthritis in 23% of the patients of this study. So, in these patients uveitis represented the first sign of JIA. Children with initial uveitis had at 5 years of follow-up significantly more frequently posterior synechiae, band keratopathy, and cystoid macular edema, but less secondary glaucoma than children with classic presentation of JIA with initial arthritis and later uveitis. Interestingly, boys presented with uveitis as initial manifestation of JIA significantly more frequently than girls (44% of boys vs 15% of girls).

In multivariate analysis male gender appeared to be independently associated with cataract surgery, cystoid macular edema and papillitis, while initial uveitis was an independent factor associated with development of posterior synechiae, which is also known as a risk factor for a complicated course of JIA-associated uveitis from the literature.

Visual outcome of boys compared to girls was also significantly worse at 1 and 3 years of follow-up. But this was also the case for children with initial uveitis before arthritis, who had significantly worse visual acuity compared to children with initial arthritis, up to 3 years of follow-up.

In the whole series, blindness, defined as best corrected visual acuity of 20/200 or worse, was identified in 10% of affected eyes in our series. This means that 15% of patients in our study were affected by uni- or bilateral blindness. Secondary glaucoma was the most common cause of visual loss in these eyes (50%). Boys' eyes were significantly more frequently affected by blindness than girls' eyes (21% vs 6%). The majority of patients with blind eyes had initial uveitis instead of arthritis (58% vs 42%) and this difference was statistically significant. However, in a multivariate analysis blindness was independently associated with male gender and not with initial uveitis.

No significant difference in development of complications and vision between younger-onset (<7 years) and older-onset (>7 years) groups was noted in this study.

We conclude that male gender and uveitis as initial manifestation of JIA are associated with a complicated course in JIA-associated uveitis. Male gender also appears to be a risk factor for poor visual prognosis in JIA-associated uveitis. Age of onset does not seem to have independent prognostic value for the course of JIA-associated uveitis. We recommend to ophthalmologists to be aware of the role of these factors and to take them in their prognostic consideration in an individual patient with JIA-associated uveitis.

In **chapter 5** we investigate the course and activity of JIA-associated uveitis in childhood and puberty in a retrospective analysis of clinical data of 62 patients with JIA and uveitis. It is remarkable that although girls with JIA are known to have a higher risk of developing uveitis, boys with JIA-associated uveitis, appear to have a more serious progression of disease, with a poorer prognosis, as described in **chapter 3-4**. The cause of these differences between genders is unknown, but it might suggest an involvement of hormonal influence.
In general, high uveitis activity was found during early years of age (4-6 years of age), followed by a quiet stage around the age of 9 and 10, after which activity rose again. This association was significantly compatible with a quadratic model. We also saw an increase in systemic medication with age in these children.
The results of this study suggest that JIA-associated uveitis encompasses a biphasic course; a high initial disease activity, followed by a quiet stage and a new wave of activity during early teenage years. This increase in uveitis activity during early teenage years was also indirectly reflected by the increased use of immunosuppressive medication in estimated puberty years in patients with JIA-associated uveitis.

Chapter 6 is a study that attempts to answer the question about the effect, optimal duration of methotrexate therapy and about the risk of relapse of uveitis after its discontinuation. In this retrospective study we assessed clinical data of 22 pediatric JIA patients, treated with methotrexate for active uveitis. Eighty-two percent of patients showed improvement of their uveitis activity with at least a 2-step decrease of the anterior chamber cells within the first year of methotrexate therapy. A topical steroid–sparing effect was observed when methotrexate was administered for a period from 3 to 9 months. Methotrexate was discontinued because of inactive uveitis in 13 patients. In sixty-nine percent of these patients a relapse of uveitis was observed after a mean time of 7.5 months after the withdrawal of methotrexate. In 46% of patients a relapse occurred within the first year after the withdrawal of methotrexate. Longer inactivity under methotrexate therapy was independently protective for relapses after the withdrawal; to be specific, 1-year increase of duration of inactive uveitis before the withdrawal of methotrexate resulted in a decrease of hazard for a new relapse of 93%. In conclusion, our study confirms the efficacy of methotrexate in the management of JIA-associated uveitis. After the withdrawal of methotrexate, the relapse of uveitis occurs in the majority of JIA patients within the first year. Our results indicate that prolonged treatment with methotrexate and especially a longer period of inactivity before the withdrawal may be desirable to minimize new relapses after the withdrawal

of methotrexate. Our results indicate that the period of inactivity before withdrawal should be preferably 2 years or longer.

In **chapter 7** we search for intraocular biomarkers in JIA-associated uveitis, using Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry (SELDI-ToF MS). Keeping in mind that uveitis can be the first sign of JIA, as described in **chapter 3-4**, and the absence of a definitive diagnostic test, there is a strong rationale for efforts to identify specific biomarkers in the ocular fluid of children with JIA-associated uveitis. We investigate protein profiles in aqueous humor (n = 73) and serum (n = 105) samples from a total of 116 children with and without uveitis. The study population includes patients with JIA-associated uveitis (definitive JIA), suspected JIA (uveitis typical for JIA, but not meeting criteria of International League against Rheumatism for diagnosis of JIA), other pediatric uveitis entities and non-inflammatory pediatric controls.
We found that in the definitive JIA group 3 protein peaks in aqueous humor at mass/charge (m/z) 6,672; 8,725 and 13,762 had qualitative and quantitative differences in expression compared to the other uveitis entities and the controls. Interestingly, definitive JIA and suspected JIA samples did not differ in their protein profiles in aqueous humor. The quantitative expression of m/z 13,762 peak in definitive and suspected JIA samples was associated with activity of uveitis. By the intraocular presence of the protein peak at m/z 13,762 definitive JIA samples could be distinguished from other uveitis entities and controls with a sensitivity of 64% and a specificity of 95%. Using this algorithm, 63% patients with suspected JIA could be clustered together with definitive JIA patients. Mass spectrometric analysis revealed the peak at m/z 13,762 as transthyretin. Increasing evidence suggests involvement of transthyretin in various biologic processes. A plentitude of isoforms have been reported for transthyretin, many of which are causally associated with formation of amyloid fibrils. Intraocular production of transthyretin by ciliary pigment epithelium has been described in a rabbit model in the literature. Considering its suggested production by ciliary pigment epithelium, local production of transthyretin in aqueous humor in our study seems plausible. In our study the ion at m/z 13,762 was not detected in serum, which might suggest its intraocular origin.
We conclude that aqueous humor of patients with JIA-associated uveitis has a distinct expression profile of proteins compared with controls and other uveitis entities. These profiles are comparable with profiles of uveitis patients suspected for JIA-association on clinical grounds, but who do not meet criteria of International League against

Rheumatism completely. These findings suggest common intraocular molecular processes in patients with JIA-associated uveitis and chronic anterior uveitis without arthritis. Transthyretin, the protein identified, could be involved in the pathogenesis of JIA-associated uveitis; however its role and the associated mechanism require further investigation.

Chapter 8 describes the immunohistochemical investigation of cellular infiltration in uveitis in JIA and other pediatric uveitis entities. Iris specimens were obtained during trabeculectomy, from 24 eyes with uveitis diagnosed before the age of 16 years and 6 eyes of children with open angle non-uveitic glaucoma. Histologically, mild to moderate signs of inflammation were found in 64% of specimens with JIA-associated uveitis while clinically only 9 % of these patients had mild uveitis activity shortly before surgery. All but 1 iris specimens from patients with JIA-associated showed a non-granulomatous type of inflammatory reaction. One of the JIA specimens was characterized by the abundance of giant cells typical for a granulomatous inflammatory process. This is a remarkable finding since uveitis in JIA is generally considered as a non-granulomatous entity. Two non-granulomatous JIA specimens with moderate inflammatory reaction were studied immunohistochemically. Both specimens showed presence of CD4+, CD68+ and CD138+ cells, while CD8+ and CD20+ cells could be detected only in one of them. Five different uveitis specimens including JIA, idiopathic anterior uveitis and idiopathic intermediate uveitis, were consistent in the presence of CD4+ T cells in the inflammatory infiltrates, while presence of other cell types in these specimens was discrepant.
We conclude that CD4+ T cells are present in inflammatory infiltrates in different childhood uveitis entities, including JIA-uveitis. Uveitis in JIA is characterized by a mixed inflammatory infiltrate in the iris with involvement of CD4+ T cells, plasma cells and histiocytes. These findings could form the basis for further research for the specification of the subsets and the roles of these cells in the pathogenesis of JIA-associated uveitis.

In **chapter 9** we investigate prognostic factors in the second most common uveitis entity in children, intermediate uveitis by retrospective analysis of clinical data of 35 children with intermediate uveitis. Visual outcomes and development of complications were analysed in relation to age of onset (< 7 and > 7 years) and ocular signs at presentation. We found that younger onset of intermediate uveitis was associated with higher risk of development of secondary glaucoma, vitreous haemorrhage and

cataract. The younger-onset group also had worse visual outcomes up to 3 years of follow-up compared to the older onset group. Unilateral legal blindness occurred in 20% of the patients, no difference in frequency of blindness was found between younger and older onset groups. Cystoid macular edema and/or maculopathy represented the main cause of blindness in this population. Development of cystoid macular edema was independently associated with papillitis and snowbanking at initial presentation. Other complications at onset were not predictive for future complications. Over time, anti-inflammatory (and immunopuppressive) therapy (topical and systemic) could be stopped completely in 69% of patients. Continuation of any kind of anti-inflammatory therapy was significantly longer and more frequently needed in children with a younger age of onset compared with the older onset group. In the group with onset of intermediate uveitis < 7 years, remission was reached 25% versus 58% in patients with older onset of intermediate uveitis.
We conclude that children with young onset of intermediate uveitis carry a higher risk of complications and worse visual outcome. Papillitis and snowbanking at initial presentation are unfavourable factors (unrelated to age of onset), associated with development of cystoid macular edema, the main cause of visual loss in pediatric intermediate uveitis.

In **chapter 10** we report on 3 pediatric cases with coexistence of idiopathic intermediate uveitis and alopecia areata. These are the first 3 cases with this coexistence of which we are aware in the literature. Alopecia areata preceded the diagnosis of bilateral intermediate uveitis in 1 child and followed within several months after intermediate uveitis diagnosis in 2 children. All 3 children were otherwise healthy. The results of extensive diagnostic evaluations were negative in all 3 cases. However, family history of all 3 children was positive for other autoimmune conditions. The severity of uveitis ranged from mild to sight-threatening, and hair loss ranged from local lesions in 2 cases to total alopecia in 1 case. The exact pathogenesis of intermediate uveitis and alopecia areata has not yet been revealed. Both conditions are considered to be autoimmune and T-cell-mediated with CD4+ cells playing a major role in their pathogenesis. Both the eye and the hair follicle are considered to be immune-privileged sites with similar mechanisms of immune privilege. So, an immune-privilege collapse or an escape from peripheral tolerance might be key factors associated with the induction of both conditions.
We conclude that theoretically, the coexistence of intermediate uveitis and alopecia areata might be based on the similarities in their complex pathogenesis. However,

more research is needed to evaluate if the coexistence is based on an association between 2 autoimmune disorders or if it is a coincidence.

In **chapter 11** we assess the effect of an Ahmed glaucoma valve implant on corneal endothelial cell density in children with uveitic glaucoma. The results of this cross-sectional study (80 eyes of 42 patients included) show that previous Ahmed glaucoma valve implantation is associated with a lower corneal endothelial cell count in eyes with secondary glaucoma due to uveitis. Endothelial cell density was significantly lower in eyes with an Ahmed glaucoma valve implant compared to eyes with uveitis without the implant. The mean time following an Ahmed glaucoma valve implantation contained 3.5 years in our study. Factors significantly associated with decreased endothelial cell density in our study included presence of an Ahmed glaucoma valve implant, previous intraocular surgery, age, duration of uveitis and history of corneal touch by the implant tube. Following a multivariate analysis, presence of an Ahmed glaucoma valve implant remained associated with decreased endothelial cell density independently from the effect of age. Importantly, within the implant group, the time interval following Ahmed glaucoma valve implantation was highly correlated with decreased corneal endothelial cell density (adjusted for age).
We conclude that Ahmed glaucoma valve implants in children with uveitic glaucoma have negative effect on endothelial cell density, and this effect is associated with the time interval following Ahmed glaucoma valve implantation. This is the first study investigating the effect of Ahmed glaucoma valve implants on endothelial cell density in children with uveitis which can therefore serve as a starting point for further prospective research in this direction.

Chapter 12 is a prospective study, investigating development of ocular complications in a cohort of 49 children within 1 year after hematopoietic stem cell transplantation (HSCT) by performing systemic ophthalmologic examinations of these children before HSCT, before leaving the HSCT unit and after 3, 6 and 12 months following HSCT. Specifically, we attempted to investigate the risk of development of viral uveitis in these immunocompromised children by performing additional examinations during systemic viral reactivations within the first year after HSCT. In our study 27% of children developed an ocular complication following HSCT. Systemic viral reactivations occurred in 41% of patients, however no new ocular abnormalities were observed during viral reactivation. New onset chorioretinal scars were observed after systemic viral reactivations in 2 patients (4%), however the etiologic relationship between the viruses

and the scars could not be confirmed in this study due to the absence of active intraocular inflammation.

Ocular complications following HSCT included: dry eye syndrome (14%), (sub)retinal hemorrhage (12%), optic disc edema (6%), chorioretinal scars (4%), vitritis (2%) and increased intraocular pressure (2%). All ocular complications, except dry eye syndrome, were detected within the first 3 months following HSCT. Time point of development of dry eye syndrome in these children varied from 3 weeks to 12 months with the median of 5 months following HSCT. In most cases the symptoms of all complications were mild and self limiting. Children with a malignant indication for HSCT had higher risk for development of ocular complications compared to children with non-malignant diseases.

We conclude that ocular complications in pediatric HSCT-patients are common, although mostly mild. The risk of viral uveitis development during systemic viral reactivations is very low, however, potential risk of vision-threatening complications in this population can not be ruled out. Based on our findings, there is no strong evidence for routine ophthalmologic screening of these patients. However, we recommend performing ophthalmologic examination pre-HSCT, in case of systemic infectious reactivations and apparently when visual symptoms are recorded. Groups of patients at high risk of ocular complications, namely patients with malignancies and children of pre-amblyopic age, deserve higher awareness.

Closing remarks and future perspectives

This thesis contains new practical insights, which could assist managing uveitis in children; as well as laboratory studies, trying to increase our fundamental understanding of this complex disease.

Clinically, we point out the negative prognostic role of male gender and its association with atypical manifestation of JIA with initial uveitis. Our findings suggest that while uveitis is more common in younger girls who are ANA+, the uveitis course is more severe in boys regardless of age or ANA status. We also try to create awareness about increasing activity of JIA-associated uveitis in puberty and high relapse chance after discontinuing the treatment with methotrexate. However, the fundamental question about the basis of gender differences in JIA-associated uveitis and its increased activity in puberty requires further research. In the future it would be interesting to investigate hormonal status in patients with JIA-associated uveitis compared to age-matched controls and to relate hormonal levels to the activity of ocular disease in these patients. Furthermore, it would be challenging to investigate intraocular levels of sex hormones and expression of sex hormone receptors in the eyes of boys and girls with JIA-associated uveitis. Future studies on intraocular cytokines and mediators of inflammation in JIA-associated uveitis could pay additional attention to the gender differences in the analysis of the results to improve our understanding of this phenomenon. Fundamental understanding of the gender differences in this disease would help us with the long-term goal of optimizing management of JIA-associated uveitis for boys and girls in an effort to protect them equally.

The negative prognostic factors are identified in intermediate uveitis in children. A clinician caring for this patient population could use this information in his or her prognostic and therapeutic considerations in an individual child with intermediate uveitis.

In another part of our work we create awareness by showing an association of an Ahmed glaucoma valve implant with an increased corneal endothelial cell loss in children with secondary glaucoma due to uveitis. At this moment this observation does not change the clinical approach to an individual patient, however, we would recommend to be aware of the long-term risk for the cornea of these patients. In our patients with an Ahmed implant we therefore perform yearly endothelial cell density monitoring. However, the exact relationship and the strength of this association and consequences in the future for these patients should be determined in a prospective study, analyzing pre-operative and postoperative endothelial cell density over several years.

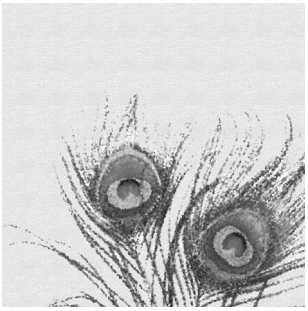
Our investigation of the cellular infiltrate in the iris is the first immunohistochemical

examination of childhood uveitis specimens not in the end-stage of the inflammatory process. Although significant discrepancies were seen between distinct JIA-specimens, CD4+ T cells, plasma cells and histiocytes were simultaneously present in the mixed inflammatory infiltrates studied immunohistochemically. For our understanding of the pathogenesis of JIA-associated uveitis it is important to know which cells infiltrate the eye in the distinct stages of the disease and to detect factors which influence the composition of the infiltrate and declare the differences between the individual patients. The identification of the infiltrating cells in the eyes with uveitis might have significant therapeutic consequences, especially with the availability of specific new biologicals. A multicenter study examining large number of trabeculectomy specimens could help us to clarify these issues.

An important finding of our study is a similar intraocular protein expression profile in JIA-associated uveitis and chronic anterior uveitis with high suspicion for JIA-association on clinical grounds. The similarity of this protein profile could on the one hand suggest that patients with suspicion for JIA-association have an atypical manifestation of JIA, which can be presented by solely chronic anterior uveitis without arthritis for several years, as we have also shown in another part of this thesis. This comparable protein profile could also indicate similar molecular processes in the eye of patients with chronic anterior uveitis in children with and without JIA. Nowadays patients with this type of uveitis are frequently being treated with immunosuppressive drugs early in the course of their disease. As these drugs form an effective treatment for JIA as well, they could mask the symptoms of arthritis in the patients with initial uveitis or even have a preventive effect for its development. However, there is no evidence for this hypothesis so far.

And last but not least, we could identify one of the three protein peaks with qualitative and quantitative differences in expression between (suspected) JIA-associated uveitis, other pediatric uveitis and controls. This protein was identified as transthyretin. Transthyretin could be involved in the pathogenesis of JIA-associated uveitis or its increased expression could be a consequence of the inflammatory sequels in these eyes. Anyhow its role and the associated mechanism require further investigation. As a variety of conditions have been associated with transthyretin recently, one should question its general value as a biomarker, although specific isoforms or posttranslationally modified forms of transthyretin could have specific roles in different (pathologic) conditions. The resolution of the SELDI-ToF MS technique did not allow for the identification of the specific transthyretin isoform of relevance in JIA, so further studies into this are warranted. It would be also interesting to perform Western blot

and ELISA analysis of transthyretin in aqueous humor and serum of children with uveitis and controls. Additionally, further research can be done on the two other protein peaks at m/z 6,672 and 8,725 which had significant qualitative and quantitative differences in (suspected) JIA compared to other uveitis and controls, but could not be identified in our study due to their low abundance, lack of material and resolution of the used methods. However, their identification could provide additional insights in our understanding of the intraocular molecular processes in JIA-associated uveitis.



Chapter 14

Samenvatting en conclusies

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Hoofdstuk 1 van dit proefschrift geeft een korte algemene introductie in de problematiek van uveitis op kinderleeftijd. Uveitis komt op kinderleeftijd relatief zelden voor, maar speelt toch een grote rol in de oogheelkundige problematiek en visuele handicap bij kinderen met deze aandoening. Door vaak voorkomende visusbedreigende complicaties kan uveitis op kinderleeftijd tot blindheid leiden. Het begint vaak asymptomatisch, echter, zelfs in vergevorderde gevallen, zijn jonge kinderen en hun ouders zich vaak niet bewust van de verminderde visus en geven de jonge kinderen er meestal geen klachten aan. Mede daardoor wordt de diagnose van uveitis op kinderleeftijd in veel gevallen pas gesteld als er reeds ernstige complicaties aanwezig zijn. Deze complicaties worden vaak al gezien bij het eerste oogheelkundige onderzoek van een kind met uveitis. Echter, vaststellen van uveitis is slechts de eerste stap in het diagnostische proces van deze aandoening. Onderliggende systemische of infectieuze oorzaken van uveitis moeten worden onderzocht. Juveniele Idiopatische Artritis (JIA) is de meest voorkomende systemische oorzaak van uveitis op kinderleeftijd en JIA-geassocieerde uveitis is tevens ook de meest voorkomende vorm van uveitis bij kinderen. JIA is verantwoordelijk voor minstens 30% van alle gevallen van uveitis bij kinderen. Echter in veel andere gevallen van kinderuveitis kan er zelfs na een uitgebreide diagnostiek geen onderliggende oorzaak gevonden worden en wordt deze uveitis idiopatisch genoemd. De tweede meest voorkomende vorm van uveitis op kinderleeftijd is intermediare uveitis, deze vorm van uveitis is bijna altijd idiopatisch op kinderleeftijd. De exacte pathogenese van een inwendige oogontsteking bij kinderen is nog niet bekend. Het wordt gezien als een auto-immune aandoening waarbij T cellen de meest belangrijke rol spleen.

Management van uveitis bij kinderen is meestal tijdrovend, complex en uitdagend. Locaal toegediende corticosteroïden vormen de eerste behandeloptie, echter een systemische behandeling met immuunsuppressiva is in veel gevallen noodzakelijk. Methotrexaat is vaak het middel van de eerste keus, vooral bij JIA patiënten. Chirurgische behandeling is vaak noodzakelijk vanwege complicaties van kinderuveitis.

Dit proefschrift is bedoeld om nieuwe inzichten te verkrijgen in het beloop en de prognostische factoren van uveitis op de kinderleeftijd **(hoofdstuk 3-5;9),** in de pathogenese van JIA-geassocieerde uveitis **(hoofdstuk 7-8)** en in de behandeling van uveitis bij kinderen **(hoofdstuk 6;11).**

Aanvullend wilden we een prospectieve studie verrichten naar oogheelkundige complicaties bij immuungecompromitteerde kinderen na een allogene haematopoietische

stamceltransplantatie (HSCT) om het risico op het ontwikkelen van virale uveitis tijdens systemische virale reactivaties te onderzoeken. Wij hebben geëvalueerd of een routinematige oogheelkundige screening noodzakelijk is bij deze patiëntenpopulatie **(hoofdstuk 12).**

Hoofdstuk 2 gaat over de complexe pathogenese van JIA-geassocieerde uveitis. Wij geven een overzicht van de literatuur en vatten de huidige kennis samen. De rollen van immuunresponsen en cytokines, genetische associaties en mogelijke externe triggers in JIA worden besproken, ondersteund door de resultaten van dierexperimentele onderzoeken naar uveitis.
Gecombineerde gegevens uit de literatuur wijzen op het ontwikkelen van abnormale immuunresponsen met betrokkenheid van zowel aangeboren ("innate") als verworven immuniteit ("adaptive"). CD4+ T cellen hebben een primaire rol in de pathogenese van JIA. Verschillende subtypes van deze cellen vervullen van zowel de initiatorfuncties (T helper (Th) 1 and Th17) als ook de regulatorfuncties van het ontstekingsproces (T regulatoire cellen (Tregs)). Er wordt verondersteld dat een disbalans tussen pro-inflammatoire Th1/Th17 cellen en anti-inflammatoire Tregs de basis van een ontstekingsreactie in JIA vormt. De rol van B cellen in de pathogenese van JIA is daarentegen minder goed onderzocht en begrepen. Het zijn vooral B cellen en plasma cellen (immunoglobulineproducerende cellen die ontstaan zijn uit B cellen) die worden beschreven als het meest rijkelijk aanwezige celtypes die het oog in JIA-geassocieerde uveitis infiltreren. Echter, deze bevindingen zijn grotendeels gebaseerd op het onderzoek van geenucleerde ogen in het laatste stadium van het ontstekingsproces en zijn mogelijk niet representatief voor de eerdere stadia van een inwendige oogontsteking in JIA. JIA-geassocieerde uveitis wordt vaak gemarkeerd door aanwezigheid van antinucleaire antistoffen. Dit wijst op de productie van abnormale autoantistoffen in deze aandoening, echter het autoantigen waartegen ze gericht zouden zijn is nog onbekend en de rol van deze autoantistoffen in de pathogenese van JIA blijft onduidelijk. JIA-geassocieerde uveitis lijkt een genetische basis te hebben, vooral geassocieerd met de human leukocyte antigen (HLA) class II. Tot nu toe is echter alleen het beschermende rol van HLA-DR1 was onafhankelijk bevestigd in meerdere onafhankelijke studies. Aan de andere kant suggereert de relatief lage concordantie van JIA-geassocieerde uveitis in tweelingen de betrokkenheid van externe (niet genetische) factoren in de pathogenese. Ondanks het feit dat veel externe factoren en infecties werden voorgesteld als triggers for JIA, werd geen enkele daarvan werd tot nu toe onafhankelijk en betrouwbaar als zodanig bevestigd. Chronische, terugkerende ontsteking in ogen

en gewrichten in JIA suggereert aanwezigheid van bepaalde overeenkomsten in deze organen, echter dit verband blijft onduidelijk.

In **hoofdstuk 3-4** analyseren wij de rol van baseline factoren voor het ontwikkelen van complicaties (**hoofdstuk 3**) and visuele uitkomsten (**hoofdstuk 4**) op de lange termijn in JIA-geassocieerde uveitis. Wij hebben een retrospectieve data-analyse uitgevoerd van 117 aangedane ogen van 65 patiënten met JIA-geassocieerde uveitis met een gemiddelde follow-up van 7.6 jaar (minimaal 1 jaar). Prognostische invloeden van geslacht, leeftijd op moment van de diagnose van uveitis en atypische manifestatie van JIA (met initiële uveitis voor artritis) werden specifiek geanalyseerd. In beide hoofdstukken laten we de associatie van mannelijk geslacht met een ongunstige prognose van JIA-geassocieerde uveitis zien. Wij vonden significant meer complicaties bij jongens vergeleken met meisjes: na 5 jaar follow-up leden de jongens vaker aan cystoid macula oedeem (50% vs 4%) en papillitis (31% vs 2%), en hadden ze frequenter cataract chirurgie nodig dan de meisjes (59% vs 32%).
In 23% van de patiënten van deze studie werd uveitis gediagnosticeerd voor artritis. Bij deze patiënten was uveitis de eerste uiting van JIA. Kinderen met initiële uveitis hadden na 5 jaar meer synechieën posterior, bandkeratopatie en cystoid macula oedeem, maar minder secundair glaucoom dan bij kinderen met klassieke manifestatie van JIA met initiële artritis en later uveitis. Opmerkelijk is dat jongens vaker uveitis hadden als initiële manifestatie van JIA significant vaker dan meisjes (44% van jongens vs 15% van meisjes).
In een multivariate analyse bleek het mannelijke geslacht onafhankelijk te zijn geassocieerd met cataract chirurgie, cystoid macula oedeem en papillitis, terwijl initiële uveitis een onafhankelijke risicofactor was voor het krijgen van synechieën posterior. Synechieën posterior zijn in de literatuur bekend als een ongunstige factor, geassocieerd met een gecompliceerd beloop van JIA-geassocieerde uveitis.
Visuele uitkomsten van jongens vergeleken met meisjes waren ook significant slechter na 1 en 3 jaar follow-up. Maar ook kinderen met initiële uveitis voor aanvang van artritis hadden een significant slechtere visus vanaf het moment van de diagnose tot en met 3 jaar follow-up, vergeleken met kinderen met initiële artritis. Blindheid werd in onze studie gevonden in 10% van de aangedane ogen. Dit betekent dat 15% van patiënten in dit onderzoek werden aangedaan door uni- of bilaterale blindheid. Secundair glaucoom was de meest voorkomende oorzaak van blindheid in deze ogen (50%). Ogen van jongens waren significant vaker getroffen door blindheid dan ogen van meisjes (21% vs 6%). De meerderheid van blinde ogen had initiële uveitis voor artritis (58% vs

42%) en dit verschil was statistisch significant. Echter, de multivariate analyse heeft uitgewezen dat blindheid onafhankelijk was geassocieerd met mannelijk geslacht en niet met initiële uveitis.
In de groepen met diagnose van uveitis op jongere of oudere kinderleeftijd (<7 jaar of >7 jaar) werd geen verschil in het ontwikkelen van complicaties gevonden.
Wij concluderen dat mannelijk geslacht en initiële uveitis als eerste manifestatie van JIA zijn geassocieerd met gecompliceerd beloop van JIA-uveitis. Mannelijk geslacht blijkt ook een risicofactor te zijn voor een slechte visuele uitkomst bij JIA-geassocieerde uveitis. Leeftijd van diagnose van uveitis in JIA lijkt geen onafhankelijke prognostische waarde te hebben in JIA-geassocieerde uveitis. Wij adviseren oogartsen om de invloed van deze factoren mee te nemen in de overwegingen bij het inschatten van de prognose van een individuele patiënt met JIA-geassocieerde uveitis.

In **hoofdstuk 5** onderzoeken we in een retrospectieve data-analyse van 62 patiënten met JIA-geassocieerde uveitis het beloop en de activiteit daarvan in kinderjaren en puberteit. Het is opmerkelijk dat ondanks het feit dat meisjes met JIA een hoger risico hebben op het krijgen van uveitis, jongens met JIA-geassocieerde uveitis een ernstiger beloop van de ziekte blijken te hebben met een slechtere prognose, zoals beschreven in **hoofdstuk 3-4**. De verklaring voor deze verschillen tussen de geslachten is onbekend, maar het zou betrokkenheid van hormonale invloeden kunnen suggereren.
Hoge uveitisactiviteit werd gevonden in de jonge kinderjaren (4-6 jaar), gevolgd door een relatief rustige fase rond 9-10 jaar met daarna een nieuwe toename van ontstekingsactiviteit. Deze associatie paste in een kwadratisch model. We laten ook zien dat gebruik van systemische medicatie in deze kinderen met de jaren stijgt.
Resultaten van dit onderzoek suggereren een bifasisch beloop van JIA-geassocieerde uveitis; een hoge initiële activiteit, dat gevolgd wordt door een relatief rustige periode en een nieuwe golf van stijgende activiteit in de tienerjaren. Deze stijging in uveitisactiviteit in de tienerjaren werd ook indirect weerspiegeld door de toename van gebruik van systemische medicatie in deze periode in onze patiënten.

Hoofdstuk 6 is een onderzoek dat naar het effect van methotrexaat. Wij onderzoeken specifiek de optimale duur van de therapie met methotrexaat en het risico van een recidief van uveitis na het stoppen van methotrexaat. In dit retrospectieve onderzoek werden klinische gegevens van 22 met methotrexaat behandelde pediatrische JIA patiënten geanalyseerd. Actieve uveitis was de reden van het initiëren van methotrexaat behandeling in alle patiënten van deze studie. Tweeëntachtig procent van

de patiënten liet een verbetering van hun uveitis activiteit laten zien met minstens 2-graad daling van de voorste oogkamer cellen binnen het eerste jaar van de therapie met methotrexaat. Een topicaal steroid-sparend effect werd geobserveerd na het gebruik van methotrexaat van 3 tot en met 9 maanden na het starten. Methotrexaat werd gestopt in 13 patiënten vanwege rustige uveitis. Negenenzestig procent van deze patiënten kreeg een recidief van uveitis na een gemiddelde periode van 7.5 maanden na het stoppen van methotrexaat. In 46% van patiënten vond een recidief plaats binnen het eerste jaar na het stoppen van methotrexaat. Langere periode van afwezige uveitis activiteit onder methotrexaat was beschermend voor het ontwikkelen van recidieven na het stoppen van methotrexaat. Om precies te zijn, ieder jaar van inactieve uveitis onder methotrexaat verminderde de kans op een recidief van uveitis na het stoppen van methotrexaat met 93%.
Concluderend, onze studie bevestigt de effectiviteit van methotrexaat in de behandeling van JIA-geassocieerde uveitis. Na het stoppen van methotrexaat kreeg bijna de helft van de patiënten binnen het eerste jaar een recidief van uveitis. Onze resultaten suggereren dat een langere behandeling met methotrexaat en vooral een langere periode van inactiviteit van uveitis voor het stoppen van methotrexaat bijdragen aan het verminderen van een recidiefkans na het stoppen van methotrexaat. Onze resultaten suggereren dat de periode van inactiviteit voor het stoppen van methotrexaat bij voorkeur minimaal 2 jaar moet zijn.

In **hoofdstuk 7** zoeken we naar biomarkers in JIA-geassocieerde uveitis. Hiervoor wordt gebruik gemaakt van Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometrie (SELDI-ToF MS). Omdat uveitis, zoals beschreven in **hoofdstuk 3-4,** de eerste manifestatie van JIA kan zijn en omdat er geen definitieve diagnostische test bestaat voor JIA, kan een onderzoek naar intraoculaire biomarkers in JIA-geassocieerde uveitis zeer zinvol zijn.
We onderzoeken eiwitprofielen in het voorsteoogkamerwater (oogvocht; n = 73) en in het serum (n =105) van total 116 kinderen met en zonder uveitis. De studiepopulatie bestaat uit patiënten met JIA-geassocieerde uveitis (definitieve JIA), suspecte JIA (uveitis beeld dat klinisch typisch is voor JIA maar niet voldoet aan de criteria van International League against Rheumatism voor de JIA diagnose), andere kinderuveitis en pediatrische niet-inflammatoire controles.
We vonden dat in de definitieve JIA groep 3 eiwitpieken in oogvocht met een gewicht/lading (mass/charge; m/z) ratio van 6,672; 8,725 en 13,762 die zowel kwalitatieve als kwantitatieve verschillen in expressie hadden, vergeleken met andere kinder-

uveitis entiteiten en controles. Een interessante bevinding daarbij is dat de definitieve JIA en de suspecte JIA monsters geen verschillen hadden in de eiwitprofielen in hun oogvocht. De kwantitatieve expressie van een m/z 13,762 piek in definitieve en suspecte JIA monsters was geassocieerd met de uveitisactiviteit. Door de intraoculaire aanwezigheid van een m/z 13,762 piek konden de definitieve JIA patiënten met 64% sensitiviteit en 95% specificiteit onderscheiden worden van andere kinderuveitis en niet-inflammatoire controls. Met hetzelfde algoritme werden 63% van de suspecte JIA patiënten geclusterd samen met de definitieve JIA patiënten. Masspectrometrie heeft m/z 13,762 piek geïdentificeerd als transthyretine. In de laatste jaren wordt gesuggereerd dat transthyretine bij veel gevarieerde biologische processen betrokken kan zijn. Er zijn veel isovormen van dit eiwit gerapporteerd, veel daarvan hebben een causaal verband met de formatie van amyloid fibrillen. Intraoculaire productie van transthyretin door het pigmentepitheel van corpus ciliare werd eerder beschreven in een konijnenmodel in de literatuur. Hierdoor lijkt een locale productie van transthyretine in oogvocht in onze studie waarschijnlijk. Ook het feit dat er in het serum geen m/z 13,762 piek werd gevonden zou een intraoculaire oorsprong kunnen suggereren.
Wij concluderen dat oogvocht van patiënten met JIA-geassocieerde uveitis een ander eiwitprofiel heeft vergeleken met controles en andere uveitiden. Dit eiwitprofiel is echter vergelijkbaar met het eiwitprofiel in het oogvocht van patiënten die uveitis hebben dat op klinische gronden suspect is voor JIA-associatie, maar die niet (volledig) voldoen aan de criteria voor de JIA diagnose. Deze bevindingen suggereren dat intraoculaire processen op moleculair niveau in patiënten met JIA-geassocieerde uveitis en patiënten met chronische uveitis anterior zonder artritis (deels) overeenkomen. Transthyretine, het geïdentificeerde eiwit, zou betrokken kunnen zijn in de pathogenese van JIA-geassocieerde uveitis; echter de specifieke rol en het daarmee geassocieerde mechanisme moeten verder worden onderzocht.

Hoofdstuk 8 beschrijft immunohistochemisch onderzoek van het ontstekingsinfiltraat in JIA-geassocieerde uveitis en andere pediatrische uveitiden. Iris monsters werden verkregen tijdens trabeculectomie van 24 ogen met uveitis gediagnosticeerd voor het 16de levensjaar en 6 ogen met open kamerhoek glaucoom zonder uveitis. Histologische tekenen van ontsteking werden gevonden in 64% van monsters met JIA-geassocieerde uveitis, terwijl klinisch maar 9% daarvan milde uveitisactiviteit had kort voor de operatie. Op één na lieten alle iris monsters van JIA-geassocieerde uveitis een beeld van een niet-granulomateuze ontsteking zien. Echter één van de JIA monsters was gekenmerkt door de aanwezigheid van vele reuscellen die typisch zijn voor een granuloma-

teus ontstekingsproces. Dit is een opmerkelijke bevinding omdat JIA (zowel uveitis als artritis) als een niet-granulomateuze ziekte wordt gezien. Twee niet-granulomateuze JIA monsters met een matige ontstekingsreactie werden immunohistochemisch bestudeerd. Beide monsters lieten aanwezigheid van CD4+, CD68+ en CD138+ cellen zien, tewijl CD8+ en CD20+ cellen alleen in 1 van deze monsters waren gedetecteerd. CD4+ T cellen en CD138+ plasma cellen waren de meest aanwezige celtypes in het infiltraat van beide JIA monsters. Vijf verschillende kinderuveitis monsters van patiënten met JIA, met idiopatische uveitis anterior en idiopatische intermediaire uveitis hadden consistent aanwezige CD4+ T cellen in het ontstekingsinfiltraat hadden, terwijl aanwezigheid van andere celltypes zeer variabel was.
Wij concluderen dat CD4+ T cellen frequent aanwezig zijn in het gemengde ontstekingsinfiltraat bij kinderuveitis. Uveitis in JIA wordt gekenmerkt door een gemengd ontstekingsinfiltraat in de iris met een betrokkenheid van CD4+ T cellen, plasma cellen en histiocyten. Deze bevindingen kunnen een basis vormen voor een verder onderzoek naar de specificaties, subtypes en rol van deze cellen in de pathogenese van JIA-geassocieerde uveitis.

In **hoofdstuk 9** onderzoeken we prognostische factoren in de tweede meest oorkomende vorm van uveitis bij kinderen, intermediare uveitis. Hiervoor gebruiken wij een retrospectieve analyse van klinische gegevens van 35 kinderen met intermediare uveitis. Visuele uitkomst en complicaties werden geanalyseerd in relatie met de leeftijd van de diagnose van uveitis (< 7 and > 7 years) en het oogheelkundige beeld bij de eerste presentatie. Wij vonden dat de diagnose van intermediatre uveitis op jonge kinderleeftijd was geassocieerd met hoger risico op het ontwikkelen van secundair glaucoom, glasvochtbloeding en cataract. De groep met de diagnose op jongere kinderleeftijd had ook slechtere visuele uitkomsten tot en met 3 jaar follow-up, vergeleken met de oudere groep. Unilaterale blindheid vond plaats in 20% van de patiënten. Frequentie van blindheid verschilde niet tussen de jongere en de oudere patiëntengroep. Cystoid macula oedeem en/of maculopatie zijn de belangrijkste redenen van blindheid in deze populatie. Het ontwikkelen van cystoid macula oedeem was onafhankelijk geassocieerd met papillitis en snowbanking bij de eerste presentatie met intermediare uveitis bij een oogarts. Aanwezigheid van andere complicaties bij de eerste presentatie was niet voorspellend voor een gecompliceerd beloop van intermediaire uveitis in de toekomst. Anti-inflammatoire en immunosuppressieve therapie kon in het beloop van intermediare uveitis volledig worden gestopt in 69% van patiënten. Het continueren van de therapie was vaker en langer noodzakelijk bij kinderen bij wie de diagnose van

intermediaire uveitis voor hun 7de jaar was gemaakt dan bij kinderen die intermediaire uveitis na hun 7de jaar hebben ontwikkeld. In de jongere groep vond remmissie van intermediare uveitis plaats in 25% van de gevallen, dit versus 58% in de oudere groep.
Wij concluderen dat kinderen met begin van intermediare uveitis op een jonge kinderleeftijd (voor 7 jaar) een hoger risico hebben op complicaties en slechtere visuele uitkomsten. Papillitis en snowbanking bij de eerste presentatie van intermediaire uveitis bij een oogarts zijn ongunstige factoren ongezien de leeftijd van de diagnose. Ze zijn geassocieerd met het later ontwikkelen van cystoid macula oedeem, de meest belangrijke oorzaak van blindheid in intermediaire uveitis bij kinderen.

In **hoofdstuk 10** beschrijven we 3 casussen van pediatrische patiënten met zowel idiopatische intermediaire uveitis als alopecia areata. Dit zijn de eerste 3 gevallen die worden beschreven in de literatuur. Bij één kind ontstond alopecia areata voor de diagnose van bilaterale intermediaire uveitis terwijl het bij de 2 andere kinderen na het begin van intermediaire uveitis begon. Het tijdsinterval tussen het ontstaan van de symptomen bedroeg enkele maanden in alle 3 de patiënten. Alle patiënten waren verder gezond. De resultaten van een uitgebreid diagnostisch proces waren negatief. Echter, de familieanamnese van alle 3 de kinderen was positief voor andere auto-immune aandoeningen. De ernst van uveitis varieerde van mild tot visusbedreigend. Het verlies van haar varieerde van locale laesies in 2 patiënten tot totale alopecia in 1 patiënt. De exacte pathogenese van zowel intermediaire uveitis als alopecia areata is nog niet ontrafeld. Beide aandoeningen worden gezien als auto-immuun, gestuurd door T cellen met name CD4+ cellen. Beiden, het oog en het haarfollikel, worden gezien als zogenaamde immuungeprivilegieerde gebieden met overeenkomende achterliggende mechanismen. Uit dit oogpunt, falen van dit immune privilege zou een belangrijke factor zijn in het ontstaan van beide aandoeningen.
Wij concluderen dat het samen voorkomen van intermediaire uveitis en alopecia areata uit het theoretisch oogpunt zou kunnen berusten op de overeenkomsten in hun complexe pathogenese. Echter, op dit moment is het niet mogelijk om te zeggen of deze observatie op een associatie tussen twee auto-immune aandoeningen berust of op een toeval.

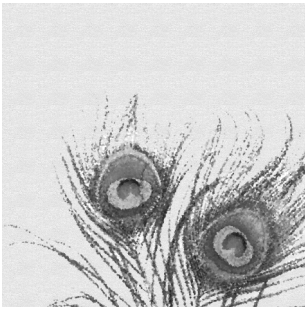
In **hoofdstuk 11** onderzoeken we het effect van een Ahmed glaucoma valve implant op de celdensiteit van het cornea endotheel bij kinderen met glaucoom als gevolg van uveitis. De resultaten van dit cross-sectionele onderzoek (80 ogen van 42 patiënten

geïncludeerd) laten zien dat een eerdere implantatie van een Ahmed implant is geassocieerd met een lagere endotheelcel dichtheid in ogen van kinderen met uveitis en secundair glaucoom.
Celdensiteit van het endotheel was significant lager in ogen met een Ahmed implant vergeleken met ogen met uveitis zonder implant. De gemiddelde tijd na het plaatsten van een Ahmed implant was 3.5 jaar in onze studie. Aanwezigheid van een Ahmed implant, eerdere intraoculaire chirurgie, oudere leeftijd, uveitisduur en een corneale touch van de Ahmed tube waren allemaal geassocieerd met een lagere endotheliale celdensiteit. In de multivariate analyse bleef het significante effect van de aanwezigheid van een Ahmed implant, zowel het effect van leeftijd, behouden. Binnen de groep ogen met een implant had het tijdsinterval na de implantatie een sterke correlatie met een lagere endotheliale celdensiteit (gecorrigeerd voor leeftijd).
Wij concluderen dat Ahmed glaucoma valve implants bij kinderen met uveitis en secundair glaucoom een negatief effect hebben op een celdensiteit van cornea endotheel en dat dit effect afhankelijk is van de tijd na de implantatie. Dit is de eerste studie die het effect van een Ahmed implant op het cornea endotheel bij kinderen met uveitis heeft onderzocht en kan als een startpunt voor verder prospectief onderzoek in deze richting worden gezien.

Hoofdstuk 12 is een prospectieve studie waarin wij oogheelkundige complicaties binnen 1 jaar na een haematopoietische stamceltransplantatie (HSCT) in een cohort van 49 kinderen onderzoeken. Wij hebben de kinderen systematisch onderzocht voorafgaande aan de HSCT, voor ontslag uit de HSCT-unit en na 3, 6 en 12 maanden follow-up. Ons doel was om specifiek het risico op het ontwikkelen van virale uveitis bij deze immuungecompromitteerde kinderen evalueren en we hebben daarom bij alle kinderen met systemische virale reactivaties aanvullende oogheelkundige onderzoeken uitgevoerd. In ons onderzoek ontwikkelden 27% van de kinderen een oculair complicatie na een HSCT. Systemische virale reactivaties vonden plaats bij 41% van de patiënten, echter geen oogheelkundige afwijkingen werden destijds gevonden. Ondanks dit feit werden er later in 2 patiënten (4%) met een voorgeschiedenis van een systemische virale reactivatie nieuwe chorioretinale littekens ontdekt. Er kon echter geen causaal verband tussen de littekens en de virussen worden aangetoond omdat er op dat moment geen sprake van een actieve intraoculaire ontsteking was.
Andere oogheelkundige complicaties in het 1e jaar na een HSCT zijn: droge ogen syndroom (14%), (sub)retinale bloeding (12%), optic disc edema (6%), chorioretinale littekens (4%), vitritis (2%) en verhoogde oogdruk (2%). Alle oogheelkundige complicaties,

behalve het droge ogen syndroom, zijn ontstaan binnen de eerste 3 maanden na een HSCT. Het moment van het ontwikkelen van droge ogen was zeer variabel (3 weken - 12 maanden na de transplantatie) met een mediaan op 5 maanden na een HSCT. De symptomen van de gevonden complicaties waren mild in de meeste gevallen. Kinderen met een maligniteit als een indicatie voor een HSCT hadden een hoger risico op oogheelkundige complicaties dan kinderen met niet-maligne indicaties voor een HSCT.

Wij concluderen dat oogheelkundige complicaties bij kinderen na een HSCT relatief vaak voorkomen, maar meestal zijn ze mild en niet ernstig. Het risico op virale uveitis tijdens systemische virale reactivaties is laag, echter een potentieel risico op het ontwikkelen van een visusbedreigende complicatie kan niet worden uitgesloten in deze populatie. In deze studie is geen sterk bewijs gevonden voor een routinematige oogheelkundige screening van deze kinderen. Toch raden wij aan om een oogheelkundig onderzoek te laten plaats vinden voor een HSCT, bij een systemische virale reactivatie en uiteraard bij klachten. Groepen van patiënten met een hoger risico, vooral patiënten met maligniteiten en kinderen van een pre-amblyope leeftijd, verdienen daarbij extra aandacht.



Dankwoord

Dankwoord

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Ook buiten de oogheelkunde heb ik belangrijke hulp gehad. Ik wil graag Marijke van

Dijk en Jaap Jan Boelens bedanken voor de prettige en vruchtbare samenwerking. Beste Marijke, bedankt voor jouw hulp aan hoofdstuk 8. Je hebt interesse in pathologie bij mij gewekt en ik heb veel van jou geleerd. Het was niet altijd even makkelijk om een gaatje te vinden in je drukke agenda, daarom extra dank voor je belangstelling en enthousiasme. Beste Jaap Jan, bedankt voor je waardevolle bijdrage aan de studie naar de oogafwijkingen bij HSCT patientjes. Extra dank voor je snelle en glasheldere communicatie!

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…we hebben gelachen, gelachen hebben
we veel en dat zal ik niet vergeten
want we hebben gelachen en veel hè?
(Bert Schierbeek)

In het laatste halfjaar werd er echter niet zo veel meer gelachen, vooral gezucht en gekreund van mijn kant. Sorry hiervoor. Maar wat is het toch heerlijk om een collega naast je te hebben die daar nooit over moppert en er nog eens voor zorgt dat je niet verhongert en uitdroogt. Dank je voor alles, en we gaan nog veel samen lachen, hè?
Lieve Ymkje, het plaatje was echter pas compleet toen jij ons kwam vergezellen en versterken. Ik keek altijd uit naar maandag en dinsdag omdat het "Ymkje dagen" waren. Je bracht een hele positieve en rustige sfeer met je mee en het viel me op dat "zuchten en kreunen" in jouw aanwezigheid vanzelf een stuk minder werd. Ik heb een grote bewondering voor jouw talent om klinische taken op twee verschillende locaties, een promotieonderzoek en drie kindjes met zo veel rust en positiviteit in goede banen te leiden. Je bent een levend bewijs dat ambitie EN rust wel degelijk samen kunnen gaan.

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Моя любимая сестра, Анечка, спасибо тебе за то что ты у меня есть и за твой хороший пример. Я очень очень очень очень сильно тобой горжусь!
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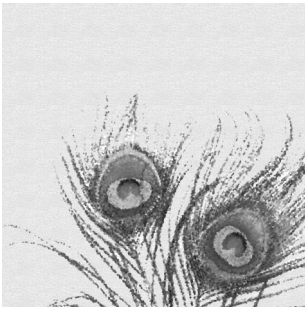
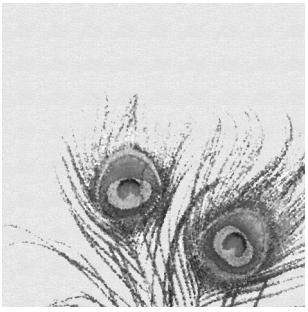


Curriculum Vitae

Curriculum Vitae

Viera Kalinina Ayuso werd geboren op 19 december 1980 te Moskou, de voormalige Sovjet Unie, waar ze ook opgroeide. Ze behaalde haar schooldiploma aan het opleidingscentrum (gymnasium-lyceum) № 109 te Moskou waar ze de laatste 3 schooljaren in een medisch-georiënteerde klas heeft gezeten. In 1998 begon ze haar studie geneeskunde aan de pediatrische faculteit van de Russische Medische Staatsuniversiteit (huidige Russische Nationale Medische Onderzoeksuniversiteit genoemd naar N.I. Pirogov) te Moskou. Na het cum laude afstuderen in 2004, verhuisde zij naar Nederland om zich bij haar Nederlandse man te voegen. Na het doorlopen van de procedure van een diploma-evaluatie, het verkrijgen van verklaring van een vakbekwaamheid en het leren van de Nederlandse taal, begon ze in 2005 aan de studie geneeskunde voor buitenlandse artsen (CIBA) aan de Universiteit Utrecht. In 2008 werd het Nederlandse artsexamen behaald. Vanaf september 2008 werkte zij als artsonderzoeker aan haar proefschrift op de afdeling Oogheelkunde van het UMC Utrecht onder begeleiding van Prof.dr. A. Rothova en dr. J.H. de Boer. Vanaf januari 2013 zal zij als arts in opleiding tot specialist werkzaam zijn op de afdeling Oogheelkunde van het UMC Utrecht. Viera is getrouwd met André Koopman en samen hebben zij een zoon, Emilian (2011).



List of publications

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1. Kalinina Ayuso V., ten Cate, HAT., van der Does P., Rothova A., de Boer JH. (2010). Male gender as a risk factor for complications in uveitis associated with juvenile idiopathic arthritis. American Journal of Ophthalmology, 149(6), 994-999.

2. Kalinina Ayuso V., ten Cate, HAT., van der Does P., Rothova A., de Boer JH. (2010). Male gender and poor visual outcome in uveitis associated with juvenile idiopathic arthritis. American Journal of Ophthalmology, 149(6), 987-993.

3. Kalinina Ayuso, ten Cate, HAT., van der Does P., Rothova A., de Boer JH. (2011). Young age as a risk factor for complicated course and visual outcome in intermediate uveitis in children. British Journal of Ophthalmology, 95(5), 646-651.

4. Kalinina Ayuso V., van de Winkel EL., Rothova A., de Boer, JH. (2011). Relapse rate of uveitis post-methotrexate treatment in juvenile idiopathic arthritis. American Journal of Ophthalmology, 151(2), 217-222.

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