# Pediatric uveitis beyond JIA

A vastitude of studied aspects on microcirculation, biomarkers, clinical course and treatment

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**Colofon**

ISBN: 978-94-6361-977-6
Design cover and chapter pages: Erwin Timmerman
Layout and print: Optima Grafische Communicatie



The research described in this thesis was financially supported by Corona Noodfonds, Oogfonds, Dr. F.P. Fischer Stichting, and ODAS Stichting.
Publication of this thesis was financially supported by Bayer, ChipSoft,Dino-Lite Europe/IDCP BV,Horus Pharma, Infection & Immunity Utrecht, Landelijke Stichting voor Blinden en Slechtzienden, Low Vision Totaal, Oculenti, Rockmed, Rotterdamse Stichting Blindenbelangen, Santen, Stichting Blindenhulp, Synga Medical, Théa Pharma, Tramedico, and Visser contactlenzen.

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## Kinderuveitis buiten het kader van JIA

Een veelvoud aan bestudeerde aspecten over microcirculatie, biomarkers, klinisch beloop en behandeling

(met een samenvatting in het Nederlands)

**Proefschrift**

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 23 mei 2024 des middags te 12.15 uur

door

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geboren op 4 september 1991
te Oss

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*Voor jullie onvoorwaardelijke steun en liefde*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AC | Anterior chamber |
| AMD | Age-related macular degeneration |
| ANA | Antinuclear antibody |
| ASUWOG | Angiography scoring for uveitis working group |
| AUC | Area under the curve |
| BCVA | Best-corrected visual acuity |
| CARRA | Childhood Arthritis and Rheumatology Research Alliance |
| CAU | Chronic anterior uveitis |
| CFH | Complement factor H |
| CI | Confidence interval |
| CME | Cystoid macular edema |
| CSCR | Central serous chorioretinopathy |
| csDMARD | Conventional synthetic disease modifying antirheumatic drug |
| EAU | Experimental autoimmune uveitis |
| EMA | European Medicines Agency |
| EULAR | European Alliance of Associations for Rheumatology |
| F13B | Coagulation factor XIII B chain |
| FA | Fluorescein angiography |
| FDA | Food and Drug Administration |
| FDR | False discovery rate |
| GEE | Generalized estimating equations |
| HLA | Human leucocyte antigen |
| HR | Hazard ratio |
| IMT | Immunomodulatory therapy |
| iCAU | Idiopathic chronic anterior uveitis |
| IF | Incidental findings |
| IL | Interleukin |
| IOP | Intraocular pressure |
| IQR | Interquartile range |
| JIA | Juvenile idiopathic arthritis |
| JIA-U | Juvenile idiopathic arthritis associated uveitis |
| LogMAR | Logarithm of the Minimum Angle of Resolution |
| LFP | Laser flare photometry |
| LRT | Likelihood ratio test |
| MMF | Mycophenolate mofetil |
| MRI | Magnetic resonance imaging |
| MTX | Methotrexate |

| | |
|---|---|
| NFC | Nailfold capillaroscopy |
| Non-JIA-U | Non juvenile idiopathic arthritis associated uveitis |
| NPX | Normalized Protein eXpression |
| OCT | Optical coherence tomography |
| OCTA | Optical coherence tomography angiography |
| OR | Odds ratio |
| PEA | Proximity extension assay |
| PH | Proportional hazard |
| QC | Quality control |
| QoL | Quality of life |
| RNFL | Retinal nerve fiber layer |
| ROC | Receiver operating characteristic |
| RPE | Retinal pigment epithelium |
| SD | Standard deviation |
| SLE | Systemic lupus erythematosus |
| SUN | Standardization of uveitis nomenclature |
| TINU | Tubulointerstitial nephritis and uveitis |
| TNF | Tumor necrosis factor |
| UMCU | University Medical Center Utrecht |
| UPOD | Utrecht Patient Oriented Database |
| VA | Visual acuity |
| VEP | Visual evoked potential |
| VFT | Visual field test |
| VKH | Vogt-Koyanagai-Harada |
| WHO | World Health Organization |

# CHAPTER 1

## General introduction and aims of this thesis

# GENERAL INTRODUCTION

Historically, uveitis is a term used to describe a diversity of inflammatory processes of the uvea. The uvea comprises of the iris, ciliary body, pars plana, and the choroid; however, any adjacent structure of the eye such as the retina, vitreous and anterior chamber can be inflamed.[1] Uveitis in children is relatively rare, accounting for 5-10% of all uveitis cases seen at tertiary referral centers, and is defined as an onset of uveitis before 16 years of age.[2] Currently, 1 in 1000 children in the Netherlands is suffering from pediatric uveitis.[3,4] Since non-infectious etiology predominates in developed countries, a large proportion of research in pediatric uveitis has been focusing on juvenile idiopathic arthritis associated uveitis (JIA-U). Non-JIA-associated uveitis (non-JIA-U) has been understudied, while still accounting for 25-60% of the total uveitis cases in children.[2,3,5,6]

Clinically, non-JIA-U can be further categorized into subgroups comprising of other systemic diseases that are associated with uveitis, including Blau syndrome and tubulointerstitial nephritis and uveitis (TINU) syndrome. Additionally, there is a significant percentage of idiopathic uveitis cases which are considered to be an isolated ocular condition. The severity of the disease frequently requires intensive systemic immunomodulatory therapy (IMT) since local treatment is not sufficient or even harmful in case of prolonged treatment to preserve vision.[7,8] In nonanterior uveitis, part of these children show inflammatory involvement of the ocular vasculature such as cystoid macular edema (CME), papillitis, retinal vasculitis, and retinal capillary leakage that negatively impact the disease course and may worsen the prognosis.[9,10] The underlying molecular mechanisms of retinal vascular involvement in pediatric uveitis remains poorly understood. Furthermore, the involvement of the systemic microcirculation has not been investigated in childhood uveitis. Investigation of microcirculation and potential biomarkers associated with vascular involvement in children with uveitis will increase our understanding of the pathophysiology of this challenging vision-threatening condition and might provide new potential biomarkers, setting a step forward towards personalized medicine in pediatric uveitis. The aim of this thesis is to get a deeper insight in the pathophysiology of pediatric uveitis and the role of microcirculation in it, to improve the current diagnostic and therapeutic strategies, give better insight in the prognosis and bring us to a more comprehensive and integrated approach in pediatric uveitis. An approach that considers different levels and aspects of this complex condition.

## Disease course

Although uveitis is rare in children, there are specific types of uveitis that are exclusively or more commonly seen in pediatric patients. Consequently, pediatric uveitis represents a distinct group of entities that often has a chronic disease course. Ophthalmologists and pediatricians face numerous challenges when dealing with this potentially vision-threatening disease. These challenges include delays in diagnosis due to the insidious and often asymptomatic nature of uveitis, difficulties in performing reliable ocular examinations due to young age, the potential risks associated with development of amblyopia, and risks of treatment side effects in growing children.[11,12] Moreover, ocular complications are more prevalent in children with uveitis compared to adults, and young children are particularly susceptible to the development of amblyopia. At time of presentation, children with uveitis may already exhibit vision-threatening complications such as posterior synechiae, band keratopathy, but also cataracts and secondary glaucoma, which may require surgical intervention.[11–13] It is likely that a child presenting with uveitis will develop one or more complications during its lifetime. Thirty-four percent of children with uveitis already present with at least one ocular complication at time of diagnosis, and 86% develop ocular complications within three years of diagnosis.[13]

Uveitis has a substantial impact on the quality of life (QoL) of both affected children and their families.[14] The QoL is influenced by stressors extending beyond visual impairment or fear of blindness, and include aspects associated with disease management. These stressors can significantly affect the psychosocial well-being. The manner in which children and families cope with these stressors exhibits variability among different patients and families. Consequently, it is of utmost importance that physicians engaged in the management of pediatric uveitis possess an understanding of how patients respond to chronic disease and its treatment. Equally crucial is an awareness of the impact of a child's disease on family dynamics and functions. Such insight is pivotal in providing comprehensive care to address not only the medical aspects but also the broader psychosocial dimensions of pediatric uveitis.

The primary goal of treating childhood uveitis is to preserve vision and prevent ocular complications while ensuring the best possible quality of life, considering that children have their entire lives ahead of them. Previous studies conducted before 2010 indicated that approximately 25-40% of pediatric uveitis patients may develop vision loss, and up to 25% may progress to legal blindness.[5,15–17] Fortunately over the past decade, there has been improvement in the rate of ocular complications and visual impairment in children with non-infectious uveitis compared to earlier studies.[18–20] This improvement can be attributed to several factors. Firstly, new therapies, including

biological agents, have emerged that can effectively control inflammation refractory to conventional immunosuppressive treatments. Secondly, specialized care provided by pediatric uveitis experts with close monitoring within a multidisciplinary team has been implemented. Lastly, in cases of JIA-U, standardized screening for uveitis has been implemented, leading to early treatment. These practices have likely contributed to better outcomes. It is important to note that these studies primarily focused on JIA-U.[18–20] Therefore, it is unclear if these results can be extrapolated to idiopathic chronic anterior uveitis (iCAU) or even to nonanterior uveitis entities. Interestingly, idiopathic CAU cannot be distinguished from JIA-U based on ophthalmological characteristics. Moreover, our research group found shared risk alleles of these two uveitis entities.[21] Therefore, it would be interesting to investigate whether iCAU exhibits a similar clinical course and visual outcomes to JIA-U in the era of biological agents, and whether these two entities of chronic anterior uveitis can be approached similarly.

## Treatment of non-infectious pediatric uveitis

Currently, despite recent and ongoing clinical trials, the treatment of non-infectious uveitis in children is primarily based on expert opinion and treatment algorithms proposed by multidisciplinary panels.[22] Guidelines and consensus treatment plans have only been established for JIA-U.[22–24] Till recently, corticosteroids were the first-line treatment for new detected uveitis to rapidly control inflammation. Nowadays, it is still frequently used for rapid control of inflammation in non-JIA-U. Prolonged use of high-dose topical corticosteroids (>3 drops daily) is associated with an increased risk of developing cataracts and secondary glaucoma.[25,26] Long-term treatment with systemic corticosteroids, even at low doses, can lead to serious side effects, including growth retardation, weight gain, hyperglycemia, increased susceptibility to infections, and osteoporosis. Consequently, the current standard practice is to transition to systemic IMT early in the course of uveitis,[7,8] and start with conventional synthetic disease modifying antirheumatic drugs (csDMARDS) such as methotrexate (MTX) and mycophenolate mofetil (MMF) as soon as possible after the diagnosis.[12,22] Unfortunately, approximately one-third of the patients on csDMARDS do not achieve sufficient disease control.[27] In such cases, the addition of a biological agent should be considered. These agents target specific molecules in the immune system that play a role in the ocular inflammatory process. Examples of biological agents include tumor necrosis factor alpha inhibitors (TNF-α inhibitors), such as adalimumab and infliximab. A recent meta-analysis demonstrated the effectiveness of adalimumab and infliximab in the treatment of chronic uveitis in children.[28] It was also demonstrated that adalimumab exhibited superior results compared to infliximab in a clinical setting. The side effect profile of adalimumab is similar to that of infliximab.[22] It should be noted that most patients included in the meta-analysis were diagnosed with JIA-U, and there-

fore data on different anatomical subtypes of uveitis are limited.[28] According to the Dutch uveitis guideline, updated on September 2020, adalimumab or infliximab are recommended as the first-choice therapy among TNF-α inhibitors for refractory non-infectious uveitis. Several factors, including patient preference, anxiety related to intravenous injections, and drug registration, play a role in the choice of treatment.[29] Studies on the effectiveness of adalimumab in non-JIA-U patients are scarce.[30,31] In Europe, adalimumab has been approved by the European Medicines Agency (EMA) for the treatment of pediatric chronic non-infectious anterior uveitis.[32] If TNF-α inhibitors fail to control the disease or if contraindications for the TNF-α inhibitors exist, tocilizumab (an interleukin-6 receptor inhibitor) is recommended. The efficacy of tocilizumab shows promise, but further studies are required to determine the optimal dose regimen.[22,33] It is recommended that all effective medications should be continued for a minimum of 2 years before considering tapering therapy.[23,34]

## Ocular microcirculation

### Vascular system of the eye

The eye possesses a complex, well-organized vascular system that facilitates appropriate oxygenation, nutrition supply, and waste removal within ocular tissues.[35,36] **Figure 1.1** provides an overview of the ocular circulation. The major arterial input to the eye is supplied by several branches originating from the ophthalmic artery, which is derived from the internal carotid artery. These branches consist of the anterior ciliary arteries, the central retinal artery, and the short and long posterior ciliary arteries.[35,36]

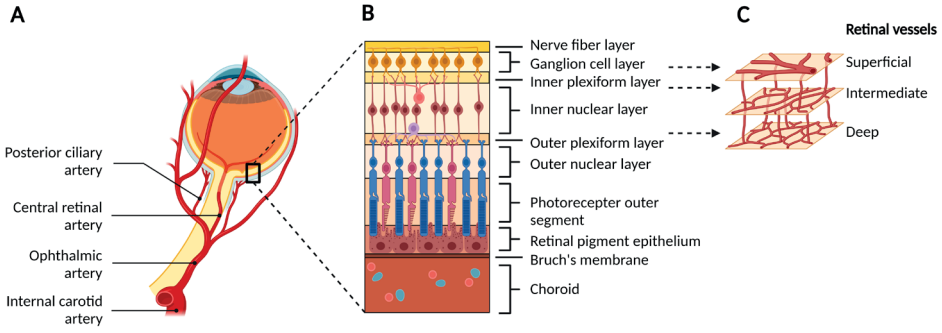


**Figure 1.1** Schematic overview of the ocular circulation. **A.** Axial image of the eyeball showing the major arterial input to the eye. **B.** The structure of the retina and the choroid. **C.** The three interconnected layers of retinal vessels: the superficial vascular plexus localized to the level of the retinal ganglion cells. The intermediate and deep capillary networks lie respectively above and below the inner nuclear layer and are supplied by vertical anastomoses from the superficial vascular plexus. Illustration created with Biorender.com.

The anterior segment of the eye, including the cornea, anterior chamber, iris, ciliary body and lens, receive blood supply from the anterior ciliary arteries that nourish the iris, and the long posterior ciliary arteries that supply the ciliary body and the choroid.[35,36] The cornea and lens are avascular, enabling light to pass through. In the absence of blood vessels, the cornea receives oxygen and nutrients that have been dissolved in the tears by diffusion. Similarly, the lens receives its nourishment for the aqueous humor via diffusion.[35]

The retinal vasculature comprises of two blood sources: (1) the central retinal artery, which enters the optic disc through an opening in the lamina cribrosa of the sclera, and (2) the choriocapillaris in the posterior part of the retina, which is derived from the short posterior ciliary arteries.[35–37] The inner part of the retina is composed of three capillary layers: the superficial, intermediate and deep layers (**Figure 1.1C**). Additionally, the peripapillary retina possesses a fourth layer adjacent to the nerve fiber layer, the radial peripapillary capillary plexus, which receives blood flow from retinal arterioles.[37] In contrast, the retinal photoreceptor layer is avascular and relies on the choriocapillaris for oxygen supply through diffusion.[35] The choroid vasculature originates from both the short posterior ciliary arteries, which supply the posterior part of the choroid, and the long posterior ciliary arteries, which supply the anterior choroid, ciliary body, and iris.[35] The choriocapillaris represents a highly fenestrated, sinusoidal vascular plexus and serves as the site of the highest blood flow in the body, accounting for up to 85% of the blood volume in the eye. Its primary function is to nourish the outer portion of retina and retinal pigment epithelium (RPE).[35]

### Imaging techniques of the ocular vasculature

Different imaging techniques are available to assess the ocular retinal vasculature. These imaging techniques are essential in the diagnosis and management of uveitis. The most commonly used techniques in pediatric uveitis include fundus photography, fundus autofluorescence, fluorescein angiography (FA), optical coherence tomography (OCT), and ultrasonography.[38] Since this thesis mainly focusses on FA and OCT, these two entities will be discussed below.

FA is a diagnostic imaging technique that involves the intravenous injection of fluorescein dye to visualize the retinal vessels.[38] A series of images is captured to assess the patency and permeability of these vessels. FA is particularly valuable in evaluating clinical markers of disease activity, including CME, retinal vascular leakage, nonperfusion, and neovascularization, see **Figure 1.2A till D**.[38–40] The Angiography Scoring for Uveitis Working Group has developed a scoring system with a good interobserver agreement, enabling standardized evaluation of FA by assessing the extent and sever-

ity of important inflammatory parameters in patients with non-inflammatory uveitis.[39] This scoring system has been used in the standardized evaluation of FA in this thesis.

While the utility of FA has been extensively studied in adults, there is a relative lack of research focusing on children.[41] This is partly due to certain disadvantages of FA in the pediatric population, such as invasiveness and the need for patient cooperation.[40,42] To overcome these challenges, oral administration of fluorescein can be used as an alternative to venipuncture, although an increased time between ingestion of the dye and imaging have to be considered.[40] Studies have demonstrated the safety of FA in children, however potential side effects may occur following dye injection (e.g., nausea, urticaria, and respiratory difficulties).[42] Recently, ultrawide field FA has been introduced. This technique enables visualization of the far periphery of the retina (from the anterior edge of vortex vein ampulla and beyond to pars plana, range of 110-220°), providing better detection of peripheral vascular leakage, peripheral nonperfusion, and neovascularization.[40,43]

Another imaging technique is optical coherence tomography (OCT) that is well-tolerated by children due to its non-invasiveness and rapid scanning speed.[40] OCT utilizes light waves of varying lengths in the near-infrared range, which travel much faster than ultrasound waves.[44] By measuring the amplitude and delay of reflected and backscattered light, OCT can provide detailed cross-sectional images of the different structures within the retina and choroid.[42] This imaging modality is widely used for evaluating the posterior segment, especially in uveitis.[40] Macular OCT is commonly used to evaluate CME (**Figure 1.2E and F)**, while measurements of the retinal nerve fiber layer (RNFL) thickness can be used to evaluate the optic disc.[40]

### Vascular abnormalities in pediatric uveitis

Inflammation usually affects the vasculature of the involved organ, as is also the case in pediatric uveitis. An overview of ocular inflammation of the posterior segment in uveitis is given in **Figure 1.3**. In pediatric uveitis vascular abnormalities include: CME, papillitis, retinal vasculitis, but also anterior chamber flare. All these clinical signs reflect the severity of uveitis; therefore, the ability to monitor these aspects at regular intervals will improve our ability to adjust the treatment course in order to minimize the risk of permanent visual impairment. The different vascular abnormalities in pediatric uveitis are discussed in detail below.

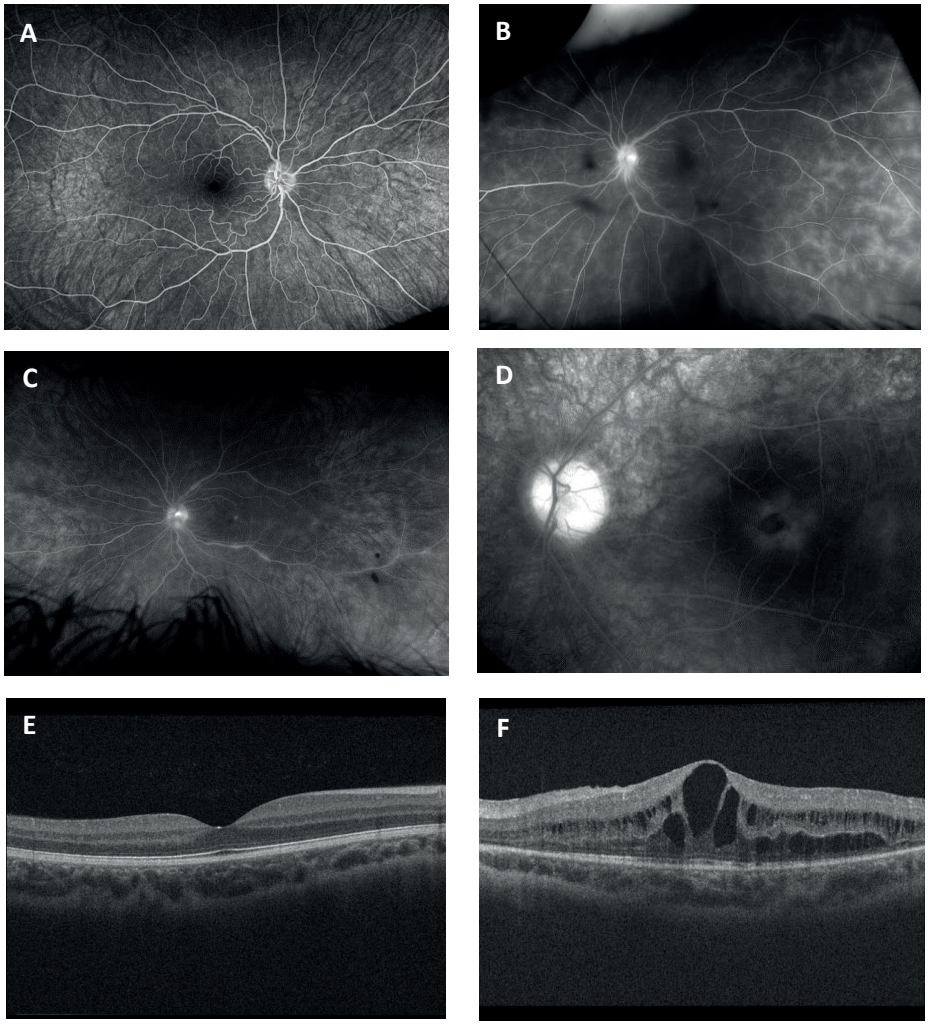


**Figure 1.2** Fluorescein angiography (FA) and optical coherence tomography (OCT) in children with non-JIA associated pediatric uveitis. **A.** FA image of a nonactive uveitis eye. **B.** FA image showing retinal capillary leakage and papillitis. **C.** FA image showing retinal vasculitis and capillary leakage **D** FA image showing cystoid macular edema and papillitis. **E.** Macular OCT of a nonactive uveitis eye. **F**. Macular OCT showing cystoid macular edema.

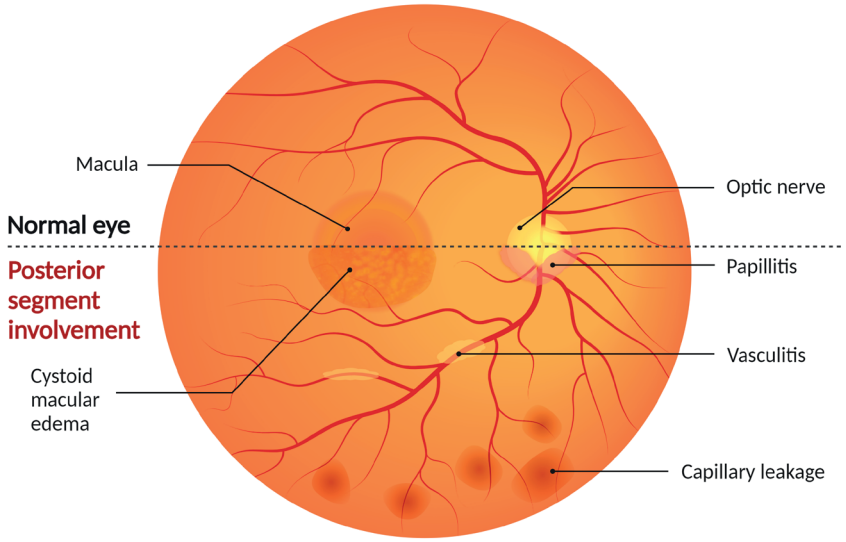


**Figure 1.3** Vascular posterior segment involvement in pediatric uveitis involving cystoid macular edema, papillitis, retinal vasculitis and capillary leakage. Illustration created with Biorender.com.

## *Anterior chamber flare*

Anterior chamber (AC) flare is an optical phenomenon caused by the breakdown of the blood-aqueous barrier due to the inflammatory process.[45,46] This breakdown causes infiltration of inflammatory cells and leakage of serum protein particles into the aqueous humor. AC flare or "Tyndall effect" can be seen during slit-lamp examination as a haze in the aqueous humor due to the reflection of protein particles.[46,47] The grading system of the *Standardization of Uveitis Nomenclature* (SUN) by slit-lamp examination is most commonly used to evaluate AC flare.[1] Laser flare photometry (LFP), a non-invasive instrument-based technique, has been accessible for over two decades and is considered superior to slit-lamp evaluation.[47] AC flare is an important clinical marker of inflammation that can be used to monitor disease progression and relapse even in the absence of inflammatory cells.[45–47] The initial flare value is found to be a strong predictor of disease course and severity. In some cases, a residual flare remains after maximal therapy. This is suggested to indicate a permanent breakdown of the blood-aqueous barrier.[47]

## *CME*

Cystoid macular edema (CME) can occur in all subtypes of pediatric uveitis. The chronic inflammation disrupts the integrity of the inner, outer or both blood-retina barriers, leading to accumulation of extracellular fluid in either the interretinal or the subretinal space.[48] Reported prevalence rates of CME in children with uveitis range from 4-30%,[5,11,15,17,20,48,49] although certain studies have reported higher rates in spe-

cific subsets of pediatric uveitis patients such as those with intermediate uveitis.[48,50] CME has been described as one of the complications with the most significant impact on visual function.[5,17,48,50] A more recent study by Eiger-Moscovich and colleagues demonstrated that CME resolved within 24 months in 76% of the cases with idiopathic pediatric uveitis.[49] Furthermore, best-corrected visual acuity (BCVA) improved at 3 months after treatment initiation. Macular OCT is considered the gold standard technique for diagnosing CME (**Figure 1.2F)**.[48] Fluorescein angiography (FA) can reveal dye diffusion in the macular area, which might be associated with pooling in the cystoid spaces (**Figure 1.2D)**.[39] Additionally, FA can provide supplementary information regarding the status of the macular vasculature and the severity of the intraocular inflammation.[39,48]

*Papillitis*

Papillitis is a less frequently studied ocular complication, even though it can occur in up to one-third of the children with uveitis.[5,11,15,16,51] Papillitis is caused by inflammation of the optic nerve head that leads to anterior swelling of the optic disc. It can be identified through fundoscopic examination; however, the examination can be complicated by many factors, including media opacities, young patient age, and a limited sensitivity of fundoscopy in milder cases. Therefore, FA is considered the gold standard for detecting papillitis, with the presence of optic disc staining and/or leakage confirming the diagnosis of papillitis (**Figure 1.2B and D**).[39] The presence of papillitis at diagnosis of uveitis is suggested to be a risk factor for developing vision-threatening complications and vision loss during follow-up.[15] Hence, it is crucial to diagnose and closely monitor papillitis in pediatric uveitis patients. In the management of uveitis, macular OCT is often used to monitor disease progression, for example in CME, and to evaluate response to treatment. However, the use of the retinal nerve fiber layer (RNFL) on OCT as an objective measure for diagnosing papillitis and evaluating response to uveitis treatment has not been fully explored. A small study involving 14 patients, including two children, demonstrated that OCT-RNFL assessment of papillitis is reliable and correlates with the presence and resolution of uveitis.[51] Nonetheless, larger studies are needed to determine if OCT-RNFL can be utilized as a non-invasive method to effectively monitor papillitis in childhood uveitis.

*Retinal vasculitis*

Retinal vasculitis is characterized by the inflammatory involvement of the retinal vessels. Currently, there are no standard criteria for diagnosis or classification of retinal vasculitis. Moreover, the SUN working group stated that consensus on the definition of retinal vasculitis required more work.[1] Taken together with the clinical heterogeneity, this makes the assessment of retinal vasculitis challenging.[40] Detection of retinal

vasculitis is made clinically, and -despite its invasiveness and time taken- confirmed through FA.[39,52] In children, the onset is insidious with minor to moderate anterior chamber reaction and vitritis. Retinal vasculitis mainly manifests as a retinal capillaritis or microvasculitis with vascular leakage at the posterior pole or peripheral retina on FA (**Figure 1.2B and C**).[9] The prevalence of retinal vasculitis is highly variable among children with idiopathic uveitis, ranging from 10% to 80%.[9,11,41,53] In a large retrospective study conducted by Yang *et al.*, involving 1364 children with idiopathic uveitis without an associated systemic disease, retinal vasculitis was observed on FA in 79.6% of the cases (77.6% of uveitis eyes). CME, on the other hand, occurred in only 9.3% of the affected eyes diagnosed on FA. The presence of retinal vasculitis was associated with a significantly higher frequency of ocular complications, such as CME, band keratopathy, and posterior or anterior synechiae. Moreover, the visual prognosis was compromised, resulting in worse visual outcomes. Thus, retinal vasculitis just as CME and papillitis is likely to be associated with active disease. Consequently, achieving inflammation control was more challenging in eyes with retinal vasculitis, and often requiring additional IMT. Therefore, Yang *et al.* recommended routine screening of children with idiopathic uveitis by FA.[9] A retrospective chart review of 14 children with intermediate or panuveitis showed similar results with 79% of the patients showing evidence for occult retinal vasculitis on FA.[53]

In another retrospective, cross-sectional study, a total of 137 eyes (*n* = 88 children with uveitis) were evaluated using FA using the standardized FA scoring system for uveitis.[39,41] Among these children, 72 patients were diagnosed with non-infectious uveitis. The FA findings revealed retinal vascular staining/leakage in 39% of the cases, capillary leakage in 70%, macular edema in 42%, and optic disc hyperfluorescence in 66% of the pediatric patients with non-infectious uveitis. It is noteworthy that the majority of these patients did not receive IMT. The utilization of FA allows for the visualization of posterior segment involvement in cases traditionally considered to primarily affect the anterior segment. In fact, several studies showed that FA detected posterior segment inflammation in up to 73% of eyes with anterior uveitis.[41,54] Note that the two here described studies of Yang *et al.* and Hossain *et al.* may be subject to selection bias, since only patients with suspected or evident vascular posterior segment involvement on clinical exam underwent FA. Therefore, the real prevalence of retinal vasculitis could be lower than described in these studies.

### Pathophysiology of vascular inflammation in pediatric uveitis

There is an urgent need for novel therapeutic interventions that are more targeted and personalized, while also minimizing side effects. To achieve this goal, a better

understanding of disease pathogenesis is crucial, especially in identifying key mediators that could serve as therapeutic targets.

The occurrence of uveitis involves multiple factors, including genetic inheritance, immune dysregulation, and environmental influences.[55] Genetic background is a significant predisposing factor for uveitis.[20,60] Various human leukocyte antigen (HLA) genes and other immune regulator genes have been identified as risk loci for uveitis.[21,56] Previously, deep HLA sequencing uncovered novel shared and disease specific HLA associations in pediatric uveitis patients.[21] With a growing understanding of the genetic predisposition, it is anticipated that future treatment strategies will be personalized and precise, based on the patient's genetic makeup.

Studying the genetic predisposition is insufficient to fully understand the complex and multifactorial disease mechanism of uveitis. For example, it is known that inflammation plays a significant role in the pathophysiology of uveitis, but the precise disease mechanisms that allow eye inflammation to occur remain very poorly understood. Various methods exist to explore the biological pathways involved in disease processes. Our research group has applied targeted array-based proteomic approaches in a cohort of non-infectious uveitis patients, especially using proximity extension array technology (PEA).[57] Targeted proteomics aims to detect and quantify relatively large panels of proteins, and multiplex arrays enable the simultaneously measurement of multiple protein analytes across large concentrations ranges, including low-abundance molecules such as chemokines and interleukins.[58,59] Application of targeted proteomics may help pinpoint biological pathways of interest by the correlation of protein analyte concentrations with the occurrence of retinal vascular involvement in cases with pediatric uveitis.

## Systemic microcirculation

Inflammatory involvement of the ocular vasculature is common in uveitis patients. We know from previous studies that the systemic immune system is activated, implying that uveitis is not exclusively an intraocular inflammation but might also be an indicator of systemic involvement.[60–65] However, whether changes in the extraocular vasculature and/or microcirculation are involved in pediatric patients with uveitis is currently unknown. A study of Chen *et al.* found nailfold microvascular abnormalities in uveitis patients that correlated with peripheral retinal leakage.[66] They believe that uveitis may be associated to systemic microvasculopathy.

Nailfold capillaroscopy (NFC) is a non-invasive imaging technique used to examine capillaries in the nailfold.[67–70] It involves the use of a capillaroscope or dermatoscope

with a magnifying lens to visualize and evaluate the morphology and microcirculation of the nailfold capillaries (see **Figure 1.4)**. During the procedure, a drop of oil or gel is applied to the nailfold area to enhance visibility. The capillaroscope is then gently pressed against the skin, allowing for direct visualization of the capillaries. The capillaries appear as fine, branching structures with distinct patterns that can be observed and analyzed. [67–69]

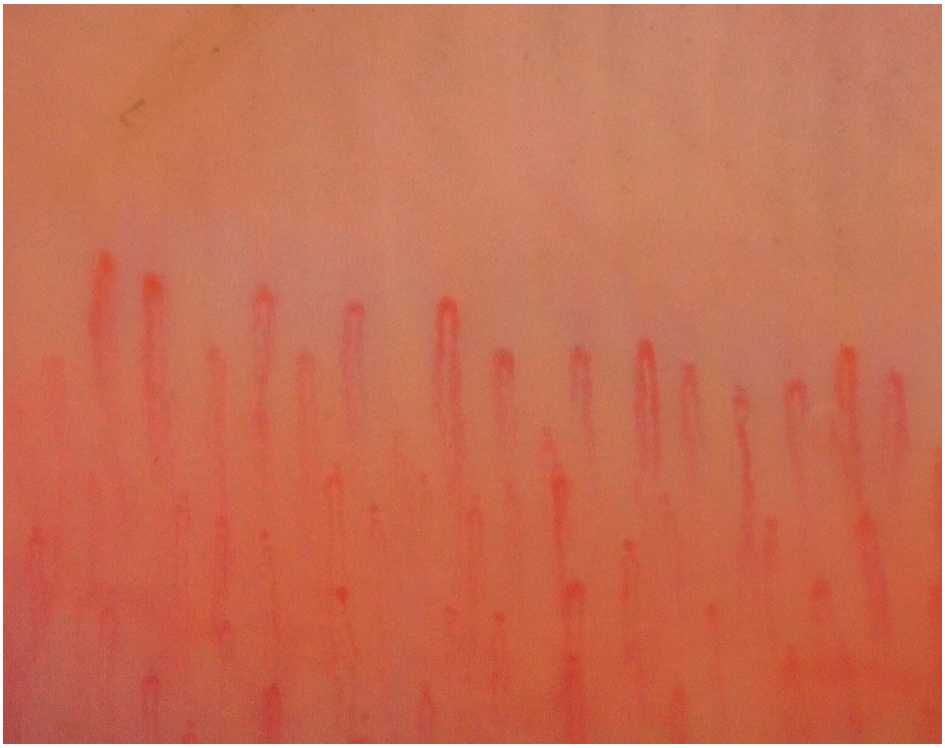

**Figure 1.4** Image of a normal aspect of capillaries in the nailfold of a healthy pediatric control made with a capillaroscope.

NFC is primarily used in the field of rheumatology to assess microvascular abnormalities associated with various systemic autoimmune diseases, particularly those involving connective tissue such as systemic sclerosis (scleroderma). The technique helps in the early detection, classification, and monitoring of these conditions, as abnormalities in the nailfold capillaries are commonly observed in patients with systemic sclerosis and Raynaud phenomenon.[67,68,70] The capillaroscopic patterns observed during nailfold capillaroscopy can provide valuable information about the health and integrity of the microcirculation, including abnormalities such as capillary dilation, capillary dropout, tortuosity, and presence of abnormal capillary shapes (e.g., giant capillaries, bushy

capillaries). These patterns can assist in the diagnosis, classification, and assessment of disease activity and severity in certain autoimmune conditions. [67–69]

NFC is a feasible examination in children because it is non-invasive, rapid, and relatively easy to perform.[69,70] However, literature on the utility of NFC in pediatric patients remains limited and heterogenous. A recent study of Melsens and colleagues presented a standardization of NFC in children and adolescents with rheumatic diseases.[68] To date, the use of NFC in children with uveitis has not been described. The standardization of the assessment of NFC in children as presented by Melsens *et al.* may, therefore, be interesting to further explore in childhood uveitis. These results may provide novel insights into the pathogenesis of this potentially vision-threatening pediatric condition, as well as providing new insights regarding the development and application of new diagnostic and/or prognostic biomarkers.

## AIMS AND OUTLINE OF THIS THESIS

The overarching aim of this thesis is to gain new insights in the pathophysiology and course of non-infectious uveitis in children without JIA, and more exploratory to investigate the role of the systemic microcirculation in pediatric uveitis. These insights will serve as a foundation for a more personalized medicine approach to pediatric uveitis and to finding new options for therapeutic targeting.

While the disease course of JIA-associated CAU is extensively studied, this is not the case for idiopathic CAU, which is the focus of **Chapter 2.** We age-matched pediatric patients diagnosed with iCAU to JIA-U patients and compared the results with regard to vision-threatening ocular complications, visual acuity and systemic treatment. Children with idiopathic uveitis can develop several vision-threatening complications during their disease course, including complications regarding posterior segment involvement.

Papillitis is an example of a complication of the posterior segment. The gold standard for diagnosing papillitis is FA, an invasive technique. Therefore, in **Chapter 3**, we investigated the diagnostic value of using OCT-RNFL, a non-invasive technique, to diagnose papillitis in pediatric uveitis. We determined an optimal cut-off value for OCT-RNFL thickness in diagnosing papillitis in children, which can serve clinicians in the therapeutic decision making.

There is a pressing need for new treatment strategies. However, the current treatment options registered for adults are not yet fully studied and approved in pediatric uveitis without JIA. The effectiveness of TNF-α inhibitors for example is mainly studied in patients with JIA-U. Moreover, the EMA only approved the use of adalimumab in pediatric patients diagnosed with anterior uveitis. In **Chapter 4** we aimed to describe the treatment results with adalimumab in all subtypes of chronic pediatric uveitis with the exception of JIA-U.

To get novel treatment insights, a better understanding of disease pathophysiology is crucial, especially in identifying key mediators that could serve as therapeutic targets. **Chapter 5** explores the biological pathways through targeted proteomics in patients with retinal vascular involvement.

Exploratory research is conducted in **Chapter 6**, where we aimed to investigate the involvement of the systemic microcirculation through nailfold capillaroscopy in patient with pediatric uveitis in order to learn more about the pathophysiology of the disease and its systemic involvement.

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# CHAPTER 2

## Clinical course and outcome in pediatric idiopathic chronic anterior uveitis

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*Am J Ophthalmol. 2022; 241:198-205. doi: 10.1016/j.ajo.2022.04.015*

## ABSTRACT

### Purpose

To examine the clinical course and outcome in children with idiopathic chronic anterior uveitis (iCAU), and to compare the results with those of age-matched children with juvenile idiopathic arthritis-associated uveitis (JIA-U).

### Design

Retrospective cohort study.

### Methods

Data regarding ocular complications, visual acuity, and systemic treatment were retrospectively collected for 2 patient groups that were matched regarding age and year of uveitis diagnosis. Outcome was evaluated using survival analysis.

### Results

The iCAU and JIA-U groups included 48 patients with 83 affected eyes and 48 patients with 73 affected eyes, respectively. Multivariate analyses showed that iCAU was associated with a higher prevalence of posterior synechiae (adjusted hazard rate [aHR] = 3.63; $P < .001$) and cataract surgery (aHR = 2.90; $P = .006$). Baseline visual acuity was worse in the iCAU group compared to the JIA-U group (20/25 vs 20/20, respectively; $P < .001$), but improved in the iCAU group after 5 years (20/20 vs 20/20, respectively; $P = .052$). At the 5-year follow-up, the younger children with iCAU (≤8 years of age at diagnosis) had a higher prevalence of posterior synechiae (aHR = 2.56; $P = .007$), secondary glaucoma (aHR = 16.0; $P = .020$), and cataract surgery (aHR = 4.79; $P = .004$) compared to older children with iCAU (≥9 years at diagnosis).

### Conclusions

Vision-threatening ocular complications are more common in children with iCAU compared to children with JIA-U, particularly in cases in which the onset of uveitis occurred at ≤8 years of age. However, the long-term vision of these children can be improved with adequate treatment.

## INTRODUCTION

Chronic anterior uveitis (CAU) is the most common form of uveitis in children. This inflammatory intraocular disease can follow a severe, debilitating course with vision-threatening ocular complications.[1–4] CAU can be associated with a variety of systemic conditions, among which juvenile idiopathic arthritis (JIA) is the most frequent and well-studied. Up to 30% of children with JIA will eventually develop CAU.[3,5] In contrast, idiopathic CAU (iCAU) accounts for approximately 30-40% of pediatric anterior uveitis cases, but remains poorly understood.[1,2,4]

No clinical distinction can be made between iCAU and juvenile idiopathic arthritis-associated uveitis (JIA-U) based on ophthalmological characteristics.[6] In addition, we recently found that these two uveitis subtypes have shared risk alleles.[7] Therefore, it is not surprising that both the Childhood Arthritis and Rheumatology Research Alliance (CARRA) standardized consensus treatment plans for comparative effectiveness research and recent therapy recommendations indicate that iCAU and JIA-U should be treated using similar strategies.[8,9]

Currently, the initial treatment for pediatric uveitis consists of topical corticosteroids. However, long-term use of topical corticosteroids can lead to sight-threatening complications such as cataracts and/or secondary glaucoma.[1,10–14] Moreover, systemic corticosteroids can have additional adverse effects on the child's general health.[15] Therefore, patients who do not respond to initial therapy, develop corticosteroid dependency, or present with a severe and/or persistent disease are typically switched to immunomodulatory therapy (IMT); however, IMT can fail in 25-50% of cases, leading to the need for additional treatment with biological agents.[16,17]

Similar to uveitis in JIA-U, uveitis in iCAU is typically asymptomatic. In the case of JIA-U, however, routine ophthalmological screening of children with JIA can often reveal the presence of asymptomatic uveitis, and early detection and treatment can help prevent ocular complications such as cataracts, secondary glaucoma, and posterior synechiae.[18] In contrast, children with iCAU and children with JIA who develop uveitis prior to the onset of arthritis are not typically subjected to routine screening for uveitis. We therefore hypothesized that children with iCAU are more likely to present with complications compared to children with JIA-U. To test this hypothesis, we conducted a retrospective cohort study in which we examined the medical records of children with iCAU and children with JIA-U and compared their clinical course, visual outcome, ocular complications, and treatment. Comparing these two patient groups can provide

important insights into whether these two types of CAU can be treated using similar approaches. These findings can also be used to guide the treatment of pediatric CAU.

## METHODS

### Study population

In this retrospective cohort study, the clinical data of pediatric patients with iCAU and a previously reported cohort of pediatric patients with JIA-U were obtained from a similar time period (from 1997 through 2020). Patients were included if they were diagnosed with uveitis before 16 years of age and had a minimum follow-up of six months. The initial diagnosis of uveitis was established by a trained uveitis specialist at the Department of Ophthalmology, University Medical Center Utrecht, in accordance with the Standardization of Uveitis Nomenclature (SUN) criteria.[19] All patients with uveitis were screened by a pediatric rheumatologist, and JIA was diagnosed in accordance with the criteria established by the International League of Associations for Rheumatology.[20,21] The patients who were diagnosed with JIA were also screened for development of uveitis in accordance with the guidelines established by the American Academy of Pediatrics.[22,23] iCAU was defined as a diagnosis of uveitis in a patient for whom JIA was not documented during the study period. All patients were seen by a pediatric rheumatologist, and patients whose CAU was caused by infection, neoplasm, or trauma were excluded. The JIA-U patients were selected from a previously reported dataset[24,25] (for patients who were diagnosed with uveitis prior to 2010) and a new dataset (with patients who were diagnosed with uveitis from 2010 through 2020) and were age-matched and matched to the date of uveitis diagnosis in the iCAU cohort. The maximum time interval of the date of uveitis diagnosis between two matched patients was two years. Matching to the date of uveitis diagnosis is performed, because literature demonstrates better visual outcomes in the "biologic era"[13] compared to earlier studies.[10] The study adhered to the tenets of the Declaration of Helsinki and was approved by the Medical Ethics Research Committee of the University Medical Center Utrecht (protocol number: 19/178).

### Data collection

Data extracted from the patients' electronic medical records included: patient demographics, uveitis characteristics, age at onset of JIA, laboratory results for antinuclear antibody (ANA) testing, ocular complications, best-corrected visual acuity (BCVA), and anti-inflammatory therapy. The following clinical data were also extracted: posterior synechiae, band keratopathy, cataract requiring surgery, secondary glaucoma, glaucoma surgery, cystoid macular edema (CME), and papillitis. Systemic treatment with

corticosteroids, IMT, and/or biological agents was also documented. Patients who received methotrexate (MTX) received a starting dose of 10-15 mg/m2/week orally or subcutaneously. Patients who were additionally treated with adalimumab received a dose of 20 mg or 40 mg (for patients weighing <30 kg or ≥30 kg, respectively) administered as a subcutaneous injection every two weeks and the MTX dose was kept at 10-15mg/m2/week. Prior to 2010, a grade of ≥1+ anterior chamber (AC) cells was used to intensify treatment in order to gain inflammatory control.[19] From 2010 and onwards, additional indicators were added, including adverse effects from topical corticosteroids at a dose of >2 drops and/or the presence of new inflammation-related complications.[8,9] Due to difficulties objectively measuring cataract formation, we used "cataract requiring surgery" to indicate the presence of cataract. Secondary glaucoma was defined using the international consensus established by the World Glaucoma Association and the Childhood Glaucoma Research Network.[26] CME was defined as the presence of macular thickening with cyst formation visible on fundoscopy, fluorescein angiography (prior to 2003), and/or macular OCT. Papillitis was defined as blurring of the optic disc margins visible on fundoscopy and/or the presence of optic disc hyperfluorescence on fluorescein angiography scored in accordance with the Angiography Scoring for Uveitis Working Group.[27] Visual impairment was defined as BCVA between 20/50 and 20/200, and legal blindness was defined either as BCVA ≤20/200 or a visual field consisting of less than 10 degrees, in accordance with the definition established by the World Health Organization (WHO).[28]

## Study outcomes

The primary outcome was the difference in the number of complications between the patients with iCAU and the patients with JIA-U at the diagnosis of uveitis and after 1 and 5 years of follow-up. The secondary outcomes were the differences in visual acuity and systemic treatment between the two groups.

## Statistical analysis

Descriptive statistics are used to report baseline characteristics and treatment, and were analyzed per patient. Except where indicated otherwise, data are represented as the median and interquartile range (IQR). Pearson's chi-squared test or Fisher's exact test was used for the univariate analysis of categorical variables to test for differences between the two groups. The Wilcoxon rank-sum test was used for medians. Snellen BCVA was converted to the logarithm of minimal angle of resolution (logMAR) for statistical analysis and converted back to Snellen BCVA for data presentation. Statistical analysis of ocular complications and BCVA were performed "by eye", including only affected eyes with 5-year follow-up data, with correction for paired eyes. A Generalized Estimating Equation (GEE) was used to adjust for the correlation between two

eyes in patients with bilateral disease (paired eyes).[29] Cox proportional hazard regression was applied in multivariate analyses, including all affected eyes with a correction of standard error with clustering using a robust method.[30] Differences with a P-value < .05 were considered significant. All tests were 2-tailed, and all statistical analyses were performed using RStudio version 4.0.3 (RStudio Inc, Boston, Massachusetts, USA).

# RESULTS

## General characteristics

A total of 48 patients with iCAU (with 83 affected eyes) and 48 age-matched patients with JIA-U (with 73 affected eyes) were included in our analysis. As shown in **Table 2.1**, the two groups did not differ significantly with respect to general patient characteristics.

**Table 2.1** General patient characteristics of the study cohorts

| | Type of CAU | | |
|---|---|---|---|
| **Patient characteristic** | **iCAU** | **JIA-U** | ***P*-value** |
| Total number of patients | 48 | 48 | |
| Total number of eyes | 83 | 73 | |
| Bilateral disease, n (%) | 35 (73) | 25 (52) | .058[a] |
| Male sex, n (%) | 14 (29) | 15 (31) | 1[a] |
| ANA-seropositive, n (%) | 30 (63) | 37 (77) | .171[a] |
| Age (years) at uveitis diagnosis, median (IQR) | 7.8 (5.9-11.1) | 7.8 (5.8-9.5) | .818[b] |
| Follow-up time (years), median (IQR) | 5.0 (2.5-8.4) | 6.1 (3.5-9.0) | .524[b] |

ANA = antinuclear antibodies, CAU = chronic anterior uveitis, iCAU, idiopathic CAU, IQR = interquartile range, JIA-U = juvenile idiopathic arthritis–associated uveitis.
[a] Pearson's chi-squared test.
[b] Wilcoxon rank-sum test.

## Ocular complications

The number and percentage of ocular complications reported at the diagnosis of uveitis and after one and five years of follow-up are summarized in **Table 2.2**. At diagnosis, the patients in the iCAU group had a generally more complicated presentation compared to the JIA-U group, with significant differences in posterior synechiae (45% vs. 14%, respectively; *P* < .001) and band keratopathy (19% vs. 7%, respectively; *P* < .05). At five years and after adjusting for the correlation of paired eyes, the patients in the iCAU group had a higher prevalence of posterior synechiae (68% vs. 31%; *P* = .002) and a higher prevalence of cataract requiring surgery (51% vs, 20%; *P* = .01);

we found no other differences between the two groups with respect to complications at the 5-year follow-up.

## Cox regression analysis

Our analysis revealed that iCAU is an independent risk factor for the need for cataract surgery (adjusted hazard ratio [aHR]: 2.90; 95% confidence interval [CI]: 1.36-6.17; *P* = .006) and for the development of posterior synechiae (aHR: 3.63; 95% CI: 2.03-6.50; *P* < .001), even after we adjusted for sex, ANA seropositivity, type of anterior uveitis (iCAU or JIA-U), and paired eyes (**Supplemental Table S2.1**). After adjusting for the other factors, we also found that an older age at diagnosis of uveitis was as-

**Table 2.2** Cumulative prevalence of ocular complications at the diagnosis of uveitis and after 1 and 5 years of follow-up

| | | Type of CAU | | |
|---|---|---|---|---|
| | Complications | iCAU | JIA-U | *P*-value |
| At diagnosis | Number of eyes | 83 | 73 | |
| | No complications | 44 (53) | 57 (78) | .001[a] |
| | Posterior synechiae, n (%) | 37 (45) | 10 (14) | <.001[a] |
| | Band keratopathy, n (%) | 16 (19) | 5 (7) | .432[a] |
| | Cataract surgery, n (%) | 0 | 0 | NA |
| | Secondary glaucoma, n (%) | 1 (1) | 1 (1) | .927[b] |
| | Glaucoma surgery, n (%) | 0 | 1 (1) | .468[b] |
| | CME, n (%) | 3 (4) | 4 (5) | .940[b] |
| | Papillitis, n (%) | 7 (8) | 6 (8) | .829[a] |
| After 1 year of follow-up | Number of eyes | 79 | 71 | |
| | No complications | 39 (49) | 39 (55) | .605[a] |
| | Posterior synechiae, n (%) | 42 (53) | 14 (20) | <.001[a] |
| | Band keratopathy, n (%) | 18 (23) | 7 (10) | .393[a] |
| | Cataract surgery, n (%) | 11 (14) | 5 (7) | .046[a] |
| | Secondary glaucoma, n (%) | 4 (5) | 1 (1) | .573[b] |
| | Glaucoma surgery, n (%) | 1 (1) | 3 (4) | .680[b] |
| | CME, n (%) | 6 (8) | 7 (10) | .979[b] |
| | Papillitis, n (%) | 8 (10) | 9 (13) | .751[a] |
| After 5 years of follow-up | Number of eyes | 41 | 51 | |
| | No complications | 14 (34) | 20 (39) | .525[a] |
| | Posterior synechiae, n (%) | 28 (68) | 16 (31) | .002[a] |
| | Band keratopathy, n (%) | 14 (34) | 11 (22) | .236[a] |
| | Cataract surgery, n (%) | 21 (51) | 10 (20) | .012[a] |
| | Secondary glaucoma, n (%) | 12 (29) | 10 (20) | .494[a] |
| | Glaucoma surgery, n (%) | 13 (32) | 12 (24) | .731[a] |
| | CME, n (%) | 8 (20) | 8 (16) | .982[a] |
| | Papillitis, n (%) | 10 (24) | 12 (20) | .951[a] |

CAU = chronic anterior uveitis, CME = cystoid macular edema, iCAU, idiopathic CAU, JIA-U = juvenile idiopathic arthritis–associated uveitis, NA = not applicable.

Analysis performed "by eye" and adjusted for correlation of paired eyes.

[a] Pearson's chi-squared test with Yates' continuity correction.

[b] Fisher's exact test.

sociated with a lower risk of cataract surgery (aHR: 0.82; 95% CI: 0.70-0.96; *P* = .013), glaucoma surgery (aHR: 0.77; 95% CI: 0.64-0.93; *P* = .006), and posterior synechiae (aHR: 0.88; 95% CI: 0.81-0.97; *P* = .006).

## Visual outcome

At the diagnosis of uveitis, BCVA was poorer in the iCAU group compared to the JIA-U group (20/25 vs. 20/20, respectively; *P* < .001 after correcting for paired eyes). We also compared the percentage of eyes that were either legally blind or visually impaired as defined by the WHO[28] and found no significant difference between groups both at baseline and after five years (**Table 2.3**). Two of the children in the iCAU group and one in the JIA-U group were treated for amblyopia during the follow-up period, and visual acuity improved slightly (from 20/125 to 20/50) for two of these children; however, both of these children developed ocular complications that compromised their vision, and therefore treatment for amblyopia was terminated.

## Treatment

Treatment with systemic corticosteroids, IMT and/or biological agents is summarized for the two groups in **Table 2.4**. At the time of their uveitis diagnosis, 19% of the children with JIA-U were already being treated with MTX for arthritis; 3 of these 9 children were also being treated with adalimumab. At five years, fewer patients in the iCAU group were being treated with MTX compared to the JIA-U group (76% vs. 97%, respectively; *P* = .037); no other significant differences were found between the two groups with respect to treatment at five years. We also found no significant difference between the two groups with respect to the interval between diagnosis and the start of MTX, with a median interval in the iCAU and JIA-U groups of 7.8 and 1.5 months, respectively (*P* = .181). Similarly, we found no significant difference with respect to the interval between diagnosis and the start of IMT (3.0 vs. 2.9 years for the iCAU and JIA-U groups, respectively; *P* = .161).

## Clinical course of pediatric iCAU based on patient age

Lastly, we compared clinical outcome between the younger children with iCAU (diagnosed at ≤8 years of age) and the relatively older children with iCAU (diagnosed at ≥9 years of age); the prevalence of ocular complications are summarized in **Supplemental Table S2.2**. We found that at the time of diagnosis, visual acuity was significantly worse in the younger group compared to the older group (median BCVA was 20/32 vs. 20/20, respectively; *P* < .001), but improved at five years and was the same in both age groups (20/20 vs. 20/20, respectively; *P* = .982); in contrast, we found no significant difference between age groups with respect to the percentage of eyes that were either legally blind or visually impaired (**Supplemental Table S2.3**). Finally, Cox

**Table 2.3.** Snellen BCVA and categorized visual outcome

| | | Type of CAU | | |
|---|---|---|---|---|
| | **Visual outcomes** | **iCAU** | **JIA-U** | ***P*-value** |
| At diagnosis | Number of eyes | 49 | 43 | |
| | BCVA, median (range) | 20/25 (20/14-20/6600) | 20/20 (20/14-20/1000) | <.001[a] |
| | ≤20/200, n (%) | 5 (10) | 2 (5) | .646[b] |
| | 20/100–20/50, n (%) | 3 (6) | 2 (5) | |
| | ≥20/40, n (%) | 41 (84) | 39 (61) | |
| After 5 years of follow-up | Number of eyes | 35 | 45 | |
| | BCVA, median (range) | 20/20 (20/14-20/100) | 20/20 (20/14-20/66) | .052[a] |
| | ≤20/200, n (%) | 0 | 0 | .314[b] |
| | 20/100–20/50, n (%) | 3 (9) | 1 (2) | |
| | ≥20/40, n (%) | 32 (91) | 44 (98) | |

BCVA = best-corrected visual acuity, CAU = chronic anterior uveitis, iCAU, idiopathic CAU, JIA-U = juvenile idiopathic arthritis–associated uveitis.
Analysis performed "by eye" and adjusted for correlation of paired eyes.
[a] Wilcoxon rank-sum test
[b] Fisher's exact test

**Table 2.4.** Treatment with systemic corticosteroids, immunomodulatory therapy and/or biological agents at diagnosis and after five years of follow-up

| | | Type of CAU | | |
|---|---|---|---|---|
| | **Treatment** | **iCAU** | **JIA-U** | ***P*-value** |
| At diagnosis | Number of patients | 48 | 48 | |
| | Systemic corticosteroids, n (%) | 0 | 0 | NA |
| | Methotrexate, n (%) | 0 | 9 (19) | .003[b] |
| | Anti-TNF-α therapy | | | |
| | Adalimumab, n (%) | 0 | 3 (6) | .242[b] |
| | Infliximab, n (%) | 0 | 0 | NA |
| After 5 years of follow-up | Number of patients | 25 | 31 | |
| | Systemic corticosteroids, n (%) | 5 (20) | 11 (35) | .245[b] |
| | Methotrexate, n (%) | 19 (76) | 30 (97) | .037[b] |
| | Anti-TNF-α therapy | | | |
| | Adalimumab, n (%) | 7 (24) | 13 (42) | .423[a] |
| | Infliximab, n (%) | 0 | 2 (6) | .497[b] |

TNF-α = tumor necrosis factor alpha, CAU = chronic anterior uveitis, iCAU, idiopathic CAU, JIA = juvenile idiopathic arthritis–associated uveitis.
[a] Pearson's chi-squared test with Yates' continuity correction.
[b] Fisher's exact test.

regression analysis showed that even after adjusting for possible confounders, being in the younger age group at the onset of uveitis was associated with a higher risk of posterior synechiae (aHR: 2.56; *P* = .007), secondary glaucoma (aHR: 16.3; *P* = .020), or cataract requiring surgery (aHR: 4.79; *P* = .004).

## DISCUSSION

In this retrospective cohort study, we found that children with iCAU have a more complicated disease course compared to children with JIA-U; specifically, they have a higher prevalence of posterior synechiae and are more likely to require cataract surgery both at the diagnosis of uveitis and during follow-up. These findings are consistent with several previous studies.[1,10,11,13] In addition, a recent cross-sectional study found that ANA-positive pediatric patients with idiopathic anterior uveitis typically present with more complications compared to patients with JIA-U at the onset of uveitis.[6] In additional analyses, we also compared the iCAU ANA-positive subgroup with the JIA-U ANA-positive subgroup and found similar results as the total group analyses. However, we found no significant differences between the iCAU ANA-negative subgroup and the JIA-U ANA-negative subgroup (data not shown), although we cannot exclude the possibility that this may be due to insufficient power, as relatively fewer patients were ANA-negative in both groups.

Interestingly, we found that being younger (≤8 years of age) at diagnosis was an additional risk factor for ocular complications in the iCAU group. Younger age at the onset of uveitis was also a prognostic factor associated with more severe uveitis in patients with JIA. In contrast, male sex is not a conclusive risk factor for visual prognosis,[1,18,24,25,31] nor was it identified as a risk factor in our iCAU cohort.

Our finding that children with iCAU are more likely to need cataract surgery compared to children with JIA-U is to be expected, given that the presence of posterior synechiae and band keratopathy at the onset of uveitis are known risk factors for cataract development,[18,32,33] and these risk factors were more common among the iCAU cohort compared to the JIA-U cohort. Cataract surgery in an eye with uveitis is a challenging procedure with potential risks to vision, particularly in young patients;[34] however, when performed in accordance with current guidelines—including extreme care during the procedure and the use of peri-operative anti-inflammatory prophylaxis—the outcome is usually good and has improved significantly in recent decades.[35–38]

Glaucoma is the most common cause of blindness among children with JIA-U.[11] Moreover, secondary glaucoma is extremely difficult to manage—particularly in young children—with a high risk of severe, irreversible visually impairment.[39,40] In our study, we found that secondary glaucoma was equally common in the two patient groups, occurring in 20-29% of patients at 5 years. Therefore, clinicians should watch monitor the occurrence of glaucoma in children with CAU—regardless of the underlying etiology—in order to minimize vision loss.

The higher prevalence of ocular complications at the diagnosis of uveitis in the iCAU group compared to the JIA-U group is likely related to the absence of screening in iCAU and the asymptomatic nature of this form of uveitis. Children diagnosed with JIA undergo regular ophthalmologic screening based on guidelines established by the American Academy of Pediatrics;[22,23] in contrast, children with iCAU do not have the possibility for screening prior to the onset of symptoms associated with ocular complications. Interestingly, up to 10% of children with JIA develop uveitis prior to arthritis;[41] we found that this subgroup of patients with JIA-U had similar baseline conditions as the patients with iCAU, as well as similar outcome with respect to ocular complications, visual acuity, and systemic treatment (data not shown).

Despite the differences in vision-threatening ocular complications between the two groups, long-term vision remained good and did not differ significantly between groups. At the diagnosis of uveitis, we found that visual acuity was significantly worse among the children with iCAU. However, even in this group median visual acuity at diagnosis was defined as "good" based on the WHO's definition;[28] thus, the statistically significant difference in visual acuity between groups may not necessarily be clinically significant, particularly given that we found no difference between groups with respect to the percentage of visual impaired or legally blind eyes. Measuring visual acuity can be challenging in young children, as their limited ability to cooperate and concentrate can influence the results.[42,43] These factors likely contributed to the differences in baseline visual acuity that we found between the younger and older age groups in the iCAU cohort. In our study, BCVA was measured in young children by specialized orthoptists, which may have led to more accurate BCVA values.

Previous studies found that the presence of ocular complications at the onset of uveitis is associated with poor visual outcome.[10,11] Nevertheless, a previous retrospective cohort study found that the differences in visual outcome between JIA-U and idiopathic non-infectious uveitis were not statistically significant.[13] These results are also consistent with the previously finding of no difference in visual acuity between groups after two years of follow-up.[6]

Nearly 20% of the children with JIA-U developed uveitis while undergoing treatment with MTX for their arthritis. MTX is the drug of choice in this population due to its efficacy and safety profile.[8,9] Indeed, during the follow-up period approximately three-quarters of the patients with iCAU and nearly all of the patients with JIA-U received MTX. However, up to 40% of patients may also require a biological agent to control the inflammation, with adalimumab as the drug of choice.[8,9] During follow-up, we found no significant difference in treatment (other than the difference in MTX), which is consistent with recent studies and published guidelines.[6,8,9]

A strength of our study is that all data were collected in a single tertiary care center for pediatric uveitis in the Netherlands, with relatively long follow-up data and uniform management practices, thus resulting in a relatively large sample size. On the other hand, our study had several limitations that warrant discussion. First, the study was retrospective. Second, we were unable to analyze the collected data regarding topical corticosteroids due to missing values such as the dose and duration. Third, the changing denominator makes it difficult to compare the groups at 1 and 5 years. However, we attempted to minimize these effects by performing Cox regression analyses.

In conclusion, our results indicate that vision-threatening ocular complications are relatively more common among children with iCAU compared to children with JIA-U. This difference is particularly evident at diagnosis and is more pronounced among children who are younger at the onset of uveitis. Despite these differences, however, long-term vision was similar between the children with iCAU and the children with JIA-U, and generally stayed good with intensive immunomodulatory therapy. Together, these findings underscore the importance of early detection and adequate treatment in order to minimize the risk of ocular complications in children with chronic anterior uveitis, regardless of the underlying cause.

## ACKNOWLEDGMENTS

Funding/Support: This study was generously supported by the Dutch Ophthalmology Foundation Oogfonds, the Dr. F.P. Fischer Stichting, and the ODAS Stichting.

Financial Disclosures: No financial disclosures.

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# SUPPLEMENTAL DATA

**Supplemental Table S2.1** Cox regression analysis of risk factors for cataract requiring surgery and the development of posterior synechiae in patients with pediatric idiopathic chronic anterior uveitis

| | Cataract requiring surgery | | | | Posterior synechiae | | | |
|---|---|---|---|---|---|---|---|---|
| | Unadjusted | | Adjusted[a] | | Unadjusted | | Adjusted[a] | |
| | Hazard ratio (95% CI) | *P*-value | Hazard ratio (95% CI) | *P*-value | Hazard ratio (95% CI) | *P*-value | Hazard ratio (95% CI) | *P*-value |
| Male sex | 1.20 (0.65-2.20) | .562 | 1.28 (0.61-2.66) | .515 | 0.76 (0.44-1.30) | .330 | 0.71 (0.37-1.38) | .318 |
| ANA seropositive | 1.13 (0.59-2.10) | .754 | 0.96 (0.42-2.18) | .923 | 1.25 (0.72-2.10) | .417 | 1.31 (0.71-2.40) | .383 |
| Age at onset | 0.85 (0.76-0.94) | .010 | 0.82 (0.70-0.96) | .013 | 0.90 (0.83-0.98) | .008 | 0.88 (0.81-0.97) | .006 |
| Idiopathic CAU | 2.53 (1.40-4.70) | .011 | 2.90 (1.36-6.17) | .006 | 3.08 (1.80-5.30) | <.001 | 3.63 (2.03-6.50) | <.001 |

ANA = antinuclear antibodies, 95% CI = 95% confidence interval, CAU = chronic anterior uveitis.

[a] Adjusted for male sex, ANA seropositive antibodies, age at onset of uveitis, type of anterior uveitis (iCAU or JIA-U), and paired eyes.

**Supplemental Table S2.2** Prevalence of ocular complications at the indicated times for the patients with idiopathic chronic anterior uveitis based on the age at diagnosis

| | | Age at uveitis diagnosis | | |
|---|---|---|---|---|
| | **Complications** | **≤8 years** | **≥9 years** | ***P*-value** |
| At diagnosis | Number of eyes | 46 | 37 | |
| | No complications | 18 (39) | 26 (70) | .009[a] |
| | Posterior synechiae, n (%) | 27 (59) | 9 (24) | .004[a] |
| | Band keratopathy, n (%) | 9 (22) | 6 (16) | .915[a] |
| | Cataract surgery, n (%) | 0 | 0 | NA |
| | Secondary glaucoma, n (%) | 0 | 0 | NA |
| | Glaucoma surgery, n (%) | 0 | 0 | NA |
| | CME, n (%) | 3 (7) | 0 | .250[b] |
| | Papillitis, n (%) | 4 (9) | 2 (5) | .687[b] |
| After 1 year of follow-up | Number of eyes | 46 | 31 | |
| | No complications | 15 (33) | 18 (58) | .048[a] |
| | Posterior synechiae, n (%) | 31 (67) | 9 (29) | .002[b] |
| | Band keratopathy, n (%) | 12 (26) | 4 (13) | .252[b] |
| | Cataract surgery, n (%) | 10 (22) | 1 (3) | .042[b] |
| | Secondary glaucoma, n (%) | 1 (2) | 3 (10) | .297[b] |
| | Glaucoma surgery, n (%) | 1 (2) | 0 | 1[b] |
| | CME, n (%) | 4 (7) | 2 (6) | 1[b] |
| | Papillitis, n (%) | 6 (13) | 1 (3) | .231[b] |
| After 5 years of follow-up | Number of eyes | 29 | 10 | |
| | No complications | 3 (10) | 5 (50) | .016[b] |
| | Posterior synechiae, n (%) | 23 (79) | 4 (40) | .043[b] |
| | Band keratopathy, n (%) | 9 (31) | 4 (40) | .704[b] |
| | Cataract surgery, n (%) | 18 (62) | 2 (20) | .031[b] |
| | Secondary glaucoma, n (%) | 12 (41) | 0 | .017[b] |
| | Glaucoma surgery, n (%) | 13 (45) | 0 | .016[b] |
| | CME, n (%) | 5 (17) | 2 (20) | 1[b] |
| | Papillitis, n (%) | 7 (24) | 2 (20) | 1[b] |

CME = cystoid macular edema, NA = not applicable.
Analysis performed "by eye" and adjusted for correlation of paired eyes.
[a] Pearson's chi-squared test with Yates' continuity correction.
[b] Fisher's exact test.

**Supplemental Table S2.3** Snellen BCVA and categorized visual outcome for the patients with idiopathic chronic anterior uveitis based on the age at diagnosis

| | | Age at uveitis diagnosis | | |
|---|---|---|---|---|
| | **Visual outcomes** | **≤8 years** | **≥9 years** | ***P*-value** |
| At diagnosis | Number of eyes | 45 | 37 | |
| | BCVA, median (range) | 20/32 (20/20-20/6300 | 20/20 (20/20-20/6300) | .004[a] |
| | ≤20/200, n (%) | 5 (11) | 1 (3) | .198[b] |
| | 20/100–20/50, n (%) | 4 (9) | 1 (3) | |
| | ≥20/40, n (%) | 36 (82) | 35 (95) | |
| After 5 years of follow-up | Number of eyes | 27 | 8 | |
| | BCVA, median (range) | 20/20 (20/14-20/100) | 20/20 (20/16-20/66) | .982[a] |
| | ≤20/200, n (%) | 0 | 0 | .553[b] |
| | 20/100–20/50, n (%) | 2 (7) | 1 (13) | |
| | ≥20/40, n (%) | 25 (93) | 7 (88) | |

BCVA = best-corrected visual acuity.
Analysis performed "by eye" and adjusted for correlation of paired eyes.
[a] Wilcoxon rank-sum test
[b] Fisher's exact test

# CHAPTER 3

## The role of the retinal nerve fiber layer thickness on OCT in the evaluation of papillitis in childhood uveitis

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*Am J Ophthalmol. 2023; 254:62-68. doi: 10.1016/j.ajo.2023.05.006*

# ABSTRACT

## Purpose

To investigate the diagnostic value of using retinal nerve fiber layer thickness measured on optical coherence tomography (OCT-RNFL) to diagnose papillitis in pediatric uveitis.

## Design

Retrospective cohort study.

## Methods

Demographic and clinical data were collected retrospectively for 257 children with uveitis (with 455 affected eyes). Receiver operating characteristic (ROC) analysis was performed to compare fluorescein angiography (FA, the diagnostic gold standard for papillitis) to OCT-RNFL in a subgroup of 93 patients. An ideal cut-off value for OCT-RNFL was then determined by calculating the highest Youden index. Finally, a multivariate analysis was applied to the clinical ophthalmological data.

## Results

Based on a subset of 93 patients who underwent both OCT-RNFL and FA, the ideal cut-off OCT-RNFL for diagnosing papillitis was >130 µm, with 79% sensitivity and 85% specificity. Among the entire cohort, the prevalence of OCT-RNFL >130 µm was 19% (27/141), 72% (26/36), and 45% (36/80) in patients with anterior uveitis, intermediate uveitis, and panuveitis, respectively. Our multivariate analysis of the clinical data revealed that OCT-RNFL >130 µm was associated with a higher prevalence of cystoid macular edema, active uveitis, and optic disc swelling on fundoscopy, with odds ratios of 5.3, 4.3, and 13.7, respectively (all $P < .001$).

## Conclusions

OCT-RNFL can be a useful noninvasive additional imaging tool for diagnosing papillitis in pediatric uveitis with relatively high sensitivity and specificity. OCT-RNFL was >130 µm in approximately one-third of all children with uveitis and was particularly prevalent in cases of intermediate uveitis and panuveitis.

## INTRODUCTION

Pediatric uveitis is an inflammatory intraocular disease that can follow a severe course and can have vision-threatening complications.[1–3] Approximately 4% of children with uveitis develop legal blindness, defined by the World Health Organization as Snellen visual acuity of 3/60 or worse.[4–6] The most common and best-studied ocular complications due to uveitis include cataracts, cystoid macular edema (CME), and secondary glaucoma, which occur in approximately 45%, 38%, and 25% of patients, respectively[1,2,7–9]; these complications develop due to intraocular inflammation, but can also occur due to the long-term effects of treatment with corticosteroids. In contrast, papillitis—inflammation of the optic nerve head resulting in swelling of the optic disc—is a relatively less-studied ocular complication, even though it can occur in up to one-third of children with uveitis.[1,2,7,8,10,11] To prevent permanent damage, papillitis must be accurately diagnosed, treated, and monitored; however, relevant studies in the context of pediatric uveitis are limited.[8,12]

Fundoscopic identification of papillitis in pediatric uveitis can be complicated by many factors, including media opacities, young patient age, and a limited sensitivity of fundoscopy in milder cases. Fluorescein angiography (FA) is therefore considered the gold standard for detecting papillitis, with the presence of optic disc staining and/or leakage confirming the diagnosis of papillitis.[13] However, in addition to being relatively invasive, FA is particularly challenging to perform in young children; therefore, a complementary non-invasive method that is rapid, widely available, and sensitive at detecting papillitis in children is clearly needed.

Optical coherence tomography (OCT) is a non-invasive imaging technique that allows for *in vivo* cross-sectional imaging of ocular tissue using light waves. Moreover, OCT has been shown to be a reliable tool for the diagnosis and management of various ocular diseases.[11,14–19] Here, we evaluated the potential of using OCT as an additional tool for diagnosing and monitoring papillitis in patients with pediatric uveitis.

## METHODS

### Study population

In this retrospective study, we included children who were diagnosed with uveitis prior to 16 years of age and had at least one retinal nerve fiber layer OCT (OCT-RNFL) thickness measurement performed between January 2010 and January 2022 (**Figure 3.1**). We excluded patients with papillitis due to hypotonia defined as intraocular pressure

<6 mmHg, optic disc drusen, malignant hypertension, and/or elevated intracranial pressure. Patients with a suspected case of optic neuritis or abnormal visual-evoked potentials with increased latencies were also excluded. All patients were diagnosed and treated at the Department of Ophthalmology, University Medical Center Utrecht. The initial diagnosis of uveitis was established by a trained uveitis specialist in accordance with the Standardization of Uveitis Nomenclature (SUN) criteria.[20] In total, 257 children with pediatric uveitis (with 455 affected eyes) were included in the analysis, including 93 children (with 167 affected eyes) who underwent both FA and OCT-RNFL with a maximum interval of three months between these two tests.

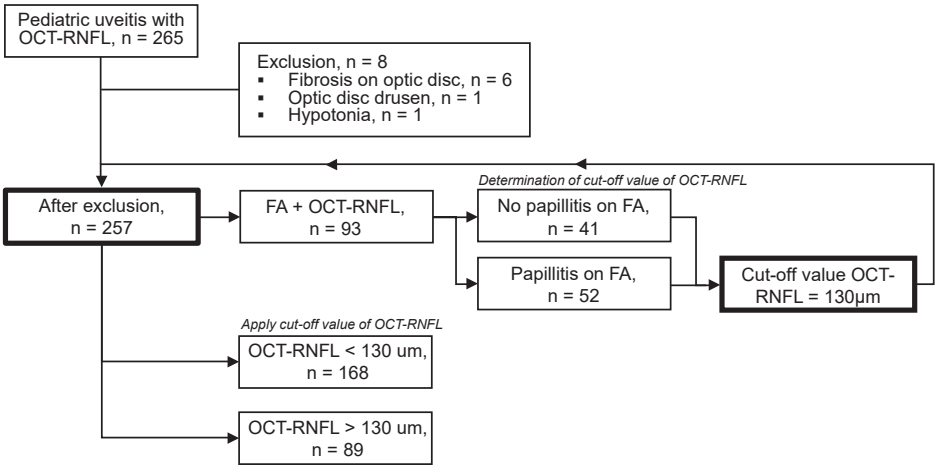


**Figure 3.1** Flowchart depicting the selection and analysis of pediatric uveitis patients. After applying the inclusion and exclusion criteria, a total of 257 patients were identified. The optimal cut-off value for OCT-RNFL thickness was first determined using fluorescein angiography (FA) as the gold standard for diagnosing papillitis (N = 93 patients). This cut-off value was then applied to the entire study cohort (N = 257 patients).

Papillitis was defined as anterior swelling of the optic disc caused by inflammation of the optic nerve head visible on FA either as diffuse staining of the disc with distinct margins or as leakage of the optic disc with blurring of the margins and the papillary vasculature, and was scored in accordance with the Angiography Scoring for Uveitis Working Group.[13] First, in the subset of 93 patients who had both FA and OCT-RNFL, the OCT-RNFL thickness values of the patients diagnosed with papillitis based on FA were compared to patients without papillitis to determine an ideal cut-off value of OCT-RNFL thickness for diagnosing papillitis using receiver operating characteristics (ROC) analysis and calculation of the highest Youden index. Next, the prevalence of patients with OCT-RNFL thickness exceeding this cut-off value was determined for the total study cohort. Finally, visual field test (VFT) results measured within three

months of OCT-RNFL were compared between the patients above and below the OCT-RNFL thickness cut-off value (corresponding to the presumed presence and absence of papillitis, respectively); for this analysis, we excluded eyes that were diagnosed with glaucoma. The study was approved by the Medical Ethics Research Committee of the University Medical Center Utrecht (protocol number: 21/147) and adhered to the tenets of the Declaration of Helsinki.



## Data collection

Data extracted from the patients' electronic medical records included: patient demographics, age at OCT measurement, uveitis subtype, the associated systemic disease, bilateral or unilateral disease, uveitis activity at OCT measurement based on the SUN criteria[20], presence of optic disc swelling on fundoscopy, presence of CME, presence of secondary glaucoma, and antinuclear antibody (ANA) serology. The following diagnostic results were also extracted: VFT, average central macular thickness (CMT) on OCT, average RNFL thickness on OCT, FA score, time interval between VFT and OCT, and time interval between FA and OCT. OCT imaging was performed using a CIRRUS HD-OCT 5000 spectral domain OCT (Carl Zeiss Meditec, Inc., Dublin, CA). CME was defined as the presence of cyst formation visible on macular OCT, and/or hyperfluoresence or leakage at the macula on FA. The presence of secondary glaucoma was used in the evaluation of the visual field examination and was defined using the international consensus established by the World Glaucoma Association and the Childhood Glaucoma Research Network.[21]

## Statistical analysis

Descriptive statistics were used to report patient characteristics and diagnostics. Binary logistic regression with a Generalized Estimating Equation (GEE) was applied for multivariate analysis to identify independent clinical ophthalmological predictive factors of papillitis. GEE analysis was also used to adjust for the correlation between both eyes in patients with bilateral disease (i.e., paired eyes).[22] We compared three models to predict whether "the eye" was diagnosed with papillitis using FA as the gold standard. The first model (model 1) consisted of OCT-RNFL thickness. The second model (model 2) was designed using only clinical ophthalmological features that were deemed to be significant in the GEE analysis. The clinical ophthalmological data used were: (1) age at the time of FA, (2) sex, (3) subtype of uveitis, (4) ANA serology, (5) active uveitis disease based on the SUN criteria[20], (6) presence of CME, and (7) presence of optic disc swelling on fundoscopy. The third model (model 3) was a combination of models 1 and 2 using both OCT-RNFL thickness and clinical data. Receiver operating characteristic (ROC) analysis was used to test the discriminative performance of the models, from which the area under the ROC curve (AUC) was calculated. The discrimi-

native performances of the ROC curves were compared as described by Delong et al.[23] The ROC curve and coordinates (sensitivity and 1-specificity scores) of model 1 were used to calculate the Youden index to determine the ideal cut-off value for OCT-RNFL thickness. Statistical analyses were performed using R version 4.0.3 (RStudio Inc., Boston, MA). The R packages "geepack" (version 1.3.4) and "pROC" (version 1.18.0) were used for GEE analysis and ROC analysis, respectively. Differences were considered significant at $P < .05$, and all tests were 2-tailed.

## RESULTS

A flowchart depicting patient selection is shown in **Figure 3.1**. The general patient characteristics of the entire study cohort (N = 257) and the subset of 93 patients with both FA and OCT-RNFL data are summarized in **Table 3.1.**

**Table 3.1** General patient characteristics of the entire pediatric study cohort and the subset of patients with a fluorescein angiography (FA) measurement.

| Per patient | Total cohort | Subset with FA |
|---|---|---|
| Total number of patients, N | 257 | 93 |
| Age (years) at OCT-RNFL measurement, mean ± SD | 10.9 ± 3.5 | 12.3 ± 2.5 |
| Male sex, N (%) | 92 (35.8) | 36 (38.7) |
| Associated systemic disease, N (%) | | |
| None | 122 (47.5) | 62 (66.7) |
| JIA | 109 (42.4) | 13 (14.0) |
| TINU syndrome | 15 (5.8) | 11 (11.8) |
| Other[a] | 11 (4.2) | 7 (7.5) |
| ANA seropositive, N (%) | 145 (56.4) | 33 (35.5) |
| **Per eye** | | |
| Total number of uveitis eyes, n | 455 | 167 |
| Active uveitis[b], n (%) | 196 (43.1) | 103 (61.7) |
| Type of uveitis, n (%) | | |
| Anterior uveitis | 236 (51.9) | 27 (16.2) |
| Intermediate uveitis | 67 (14.7) | 44 (26.3) |
| Panuveitis | 152 (33.4) | 96 (57.5) |
| Cystoid macular edema, n (%) | 43 (9.3) | 28 (16.8) |
| OCT-RNFL thickness in µm, median (IQR) | 109 (98-133) | 121 (103-143) |

ANA = Antinuclear Antibodies, IQR= Interquartile Range, JIA = Juvenile Idiopathic Arthritis, OCT-RNFL = Retinal Nerve Fiber Layer on Optical Coherence Tomography, SD = Standard Deviation, TINU = Tubulointerstitial Nephritis and Uveitis.

[a] Vogt-Koyanagi-Harada disease (*N* = 5), M. Crohn (*N* = 2), Psoriatic Arthritis (*N* = 1), Blau syndrome (*N* = 1), Eosinophilic Granulomatosis with Polyangiitis (EGPA) (*N* = 1), M. Hashimoto (*N* = 1).

[b] Active uveitis according to the Standardization of Uveitis Nomenclature (SUN) criteria.[20]

## Ideal cut-off value for OCT-RNFL thickness

First, we analyzed the subset of 93 patients (with 167 affected eyes) with both FA and OCT-RNFL measurements. The median OCT-RNFL thickness was 121 µm, with an interquartile range (IQR) of 105-143 µm. We found that OCT-RNFL thickness differed significantly between the subtypes of uveitis ($P$ = .002), with intermediate uveitis eyes having the highest thickness (median: 133 µm; IQR: 122-145 µm), followed by panuveitis (median: 118 µm; IQR: 104-140 µm) and anterior uveitis (thickness: 108 µm; 100-123 µm). Importantly, we also found that eyes with papillitis (based on FA) had significantly higher OCT-RNFL thickness (median: 148 µm; IQR: 133-177 µm) compared to eyes without papillitis (median: 109 µm; IQR: 100-123 µm; $P$ < .001). The ideal cut-off value of the average OCT-RNFL thickness was then used to differentiate between the presence and absence of papillitis using the ROC curve of model 1 (OCT-RNFL), with FA as the gold standard for diagnosis papillitis (model 1 in **Figure 3.2**). The optimal sensitivity (79%) and specificity (85%) corresponded to the highest Youden index, yielding a cut-off value for average OCT-RNFL thickness of 130 µm. The corresponding positive predictive value was 77%, and the corresponding negative predictive value was 87%, resulting in a diagnostic accuracy of 83%.

## Prediction models

Our univariate analysis of clinical ophthalmological data showed that both the presence of optic disc swelling on fundoscopy ($P$ = .001) and the presence of CME ($P$ = .01) were associated with a diagnosis of papillitis. Our multivariate binary logistic regression model with adjustment for paired eyes (GEE analysis) showed that CME (odds ratio [OR]: 5.3), active uveitis (OR: 4.3), and the presence of optic disc swelling on fundoscopy (OR: 13.7) were significant independent predictors of papillitis ($P$ < .001).

The ROC curves of the three models using FA as the gold standard are shown in **Figure 3.2**. The AUC of the first model (using the OCT-RNFL values) was 89%; in contrast, the AUCs of model 2 (using clinical data) and model 3 (using both OCT-RNFL thickness and clinical data) were 82% and 91%, respectively. Delong's test for correlated ROC curves showed significant improvement for model 1 vs. 2 ($P$ = .02), model 3 vs. model 1 ($P$ = .04), and model 3 vs. model 2 ($P$ < .001).

## Applying the cut-off value for OCT-RNFL thickness to the entire study cohort

Next, we applied the cut-off value for OCT-RNFL thickness of 130 µm (calculated based on the subgroup of 93 patients) to the entire study cohort of 257 patients **(Figure 3.1)**, revealing OCT-RNFL thickness >130 µm in 89 patients (35%). Specifically, 19% (27/141), 72% (26/36), and 45% (36/80) of patients with anterior uveitis, intermedi-

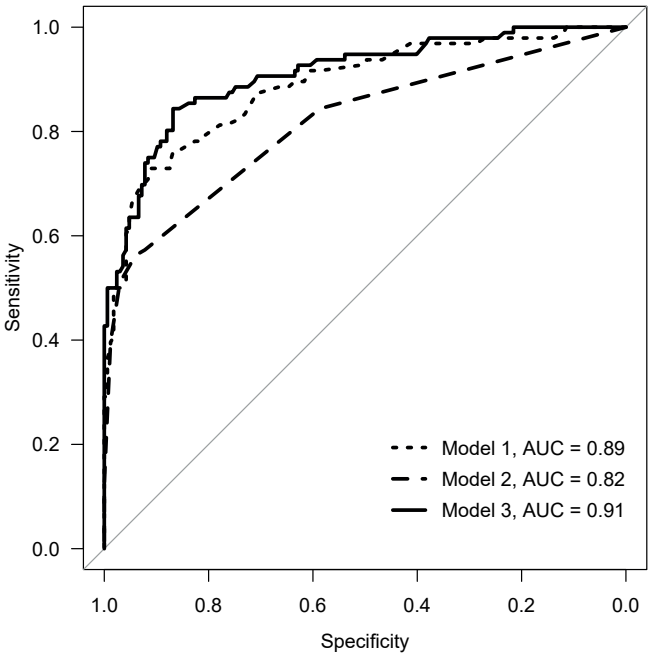


**Figure 3.2** Receiver operating characteristics (ROC) curves for predicting papillitis in pediatric uveitis using three prediction models (see Methods for details) based on Fluorescein angiography (FA) as the gold standard for diagnosing papillitis, with the corresponding area under the curve (AUC). Model 1 is based on retinal nerve fiber layer (RNFL) thickness on OCT. Model 2 is based on clinical ophthalmological data, including the presence of cystoid macular edema, active uveitis, and/or optic disc swelling on fundoscopy. Finally, model 3 is based on a combination of OCT-RNFL thickness and clinical data (i.e., models 1 and 2, respectively). All three models were adjusted for the correlation between paired eyes in bilateral cases.

ate uveitis, and panuveitis, respectively, had an OCT-RNFL thickness >130 µm. The prevalence of OCT-RNFL thickness >130 µm was also higher in patients with active uveitis (53%, $P < .001$) and patients with CME (73%, $P = .004$). The median OCT-RNFL thickness for the 196 eyes with active uveitis and was 129 µm (IQR: 107-162 µm) compared to 103 µm (IQR: 94-114 µm) for the 259 eyes without active uveitis ($P < .001$). The median OCT-RNFL thickness values were higher in all four quadrants of the OCT-RNFL measurements in the eyes with active uveitis compared to the eyes without active uveitis (all quadrants, $P < .001$).

## Visual field test

Lastly, we analyzed the VFT results for 42 eyes with OCT-RNFL thickness >130 µm and 88 eyes with OCT-RNFL thickness ≤130 µm. The most common visual field defect in the eyes with OCT-RNFL thickness >130 µm was an enlarged blind spot, present in 40% of eyes (17/42) compared to only 7% (6/88) of the eyes with OCT-RNFL thick-

ness ≤130 μm ($P$ < .001). We also found a significantly higher prevalence of normal VFT results among the eyes with OCT-RNFL thickness ≤130 μm compared to the eyes with OCT-RNFL thickness >130 μm (47% vs. 24%, respectively; $P$ = .02). No other visual field defects differed significantly between the groups (data not shown).



## DISCUSSION

This retrospective study shows that RNFL thickness measured using OCT has high sensitivity and specificity for diagnosing papillitis in pediatric uveitis. Moreover, adding clinical data such as the presence of CME, active uveitis, and optic disc swelling on fundoscopy to the prediction model based on OCT-RNFL values significantly improved the model's ability to diagnose papillitis in pediatric uveitis. Therefore, these clinical features and the findings of a thorough ophthalmological examination should be taken into account to obtain an accurate differential diagnosis and management strategy. Papillitis is one of the clinical signs reflecting the activity and severity of uveitis; therefore, the ability to monitor papillitis at regular intervals will improve our ability to adjust the treatment course in order to minimize the risk of permanent visual impairment. Although RNFL thickness measured on OCT likely cannot replace FA as the gold standard for visualizing disease activity in uveitis, it is non-invasive, fast, easy to perform, and provides quantifiable measurements, providing added value with respect to disease monitoring.

Normative data regarding RNFL and optic nerve parameters are not currently integrated in OCT devices for use in patients under 18 years of age; therefore, caution should be taken when attempting to extrapolate normative data for adults to children. Previous studies found that average RNFL thickness in healthy children can range from 98 to 108 μm[24–29], which is similar to the average RNFL thickness measured in our subgroup of patients without papillitis. Nevertheless, these thickness values were measured using different OCT devices, and variability can occur between OCT devices. Consistent with most studies involving healthy children[26,28,29], we also found no effect of age on average RNFL thickness; interestingly, one study found an apparent inverse correlation between age and RNFL thickness, but this correlation was no longer significant after they corrected for refraction.[27]

In our study cohort, the prevalence of papillitis was higher than in previous reports[1,2,7–11] and was most prevalent among patients with intermediate uveitis. In previous studies, the reported prevalence of papillitis among children with intermediate uveitis was highly variable, ranging from as low as 2% to as high as 71%. In contrast, two studies

conducted before the OCT era and at the same center yielded results similar to our current findings with respect to intermediate uveitis.[30,31] Importantly, not all previous studies included a systematic evaluation using FA; thus, the prevalence of papillitis may have been underestimated in these studies[1,2,8–10], as mild cases of papillitis can easily be missed on fundoscopy. Indeed, in our study nearly one-third of the eyes with papillitis based on FA had normal optic disc appearance on fundoscopy.

Consistent with several previous studies[8,30–32], we found that papillitis was associated with the presence or development of CME, particularly in cases of intermediate uveitis. In addition, Moore et al. reported significantly higher RNFL thickness in patients with active uveitis compared to patients with quiescent disease.[33] Both CME and papillitis are ocular complications associated with a more severe disease course; thus, the presence of one or more of these complications could be an indication to intensify treatment in an attempt to preserve vision in pediatric uveitis.

Among our patients with OCT-RNFL thickness >130 µm, the most common visual field defect was an enlarged blind spot, present in 40% of these eyes, significantly higher than in the eyes with normal OCT-RNFL thickness. In contrast, the most common visual field defect in children with optic neuritis is a central or cecocentral scotoma, which was previously reported in 50% of children who underwent visual field testing.[12]

A limitation of this study is its retrospective design, which may have resulted in missing data due to a lack of documentation in the patients' electronic medical records, particularly older records in which fundoscopy was described briefly in a limited number of cases. In addition, OCT-RNFL thickness in our cohort was measured using one specific type of OCT device. Because differences in RNFL thickness measurements can occur between different devices, our quantitative data are applicable to this specific device, and the cut-off value may differ slightly when using a different type of OCT device. Nevertheless, we do not expect major differences in cut-off values when using other devices, as most devices use a predefined diameter of 3.4-3.5 mm.[34]

In conclusion, our data suggest that RNFL thickness measured using OCT is a sensitive and specific non-invasive imaging tool for diagnosing and monitoring papillitis in various clinical types of pediatric uveitis. Based on our subgroup analysis, we determined an ideal cut-off value for RNFL thickness of 130 µm to tentatively diagnose papillitis, and we found that this cut-off value is exceeded in approximately one-third of children with uveitis and is particularly prevalent among intermediate uveitis and panuveitis. Moreover, we found that high RNFL thickness was correlated to uveitis disease activity and may be accompanied by visual field defects.

## ACKNOWLEDGMENTS

Funding/Support: This study was generously supported by the Dutch Ophthalmology Foundation Oogfonds, the Dr. F.P. Fischer Stichting, and the ODAS Stichting.

Financial Disclosures: No financial disclosures.

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# CHAPTER 4

## Clinical benefits and potential risks of adalimumab in non-JIA chronic pediatric uveitis

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*Acta Ophthalmol. 2022;100(4):e994-e1001. doi: 10.1111/aos.15012*

# ABSTRACT

## Purpose

To describe the treatment results with adalimumab in chronic paediatric uveitis, not associated with juvenile idiopathic arthritis (JIA).

## Methods

Medical records of children with non-JIA-uveitis were reviewed retrospectively. Children without an underlying systemic disease were pre-screened with brain magnetic resonance imaging (MRI) to exclude white matter abnormalities/demyelination.

## Results

Twenty-six patients were pre-screened with brain MRI, of whom adalimumab was contraindicated in six patients (23%) with non-anterior uveitis. Forty-three patients (81 eyes) were included. Disease inactivity was achieved in 91% of the patients after a median of three months (3–33). Best-corrected visual acuity (BCVA) improved from 0.16 ± 0.55 logarithm of the minimum angle of resolution (logMAR) at baseline to 0.05 ± 0.19 logMAR at 24 months ($P = 0.015$). The median dosage of systemic corticosteroids was reduced to 0 mg/day at 24 months of follow-up (versus 10 mg/day at baseline; $P < 0.001$). Adalimumab was discontinued in thirteen children due to ineffectiveness ($n = 8$), side effects ($n = 1$), long-term inactivity of uveitis ($n = 3$) or own initiative ($n = 1$). Relapse of uveitis occurred in 19 (49%) patients, 5 (26%) of them without an identifiable cause.

## Conclusion

Adalimumab is effective in the treatment of non-JIA-uveitis in paediatric patients by achieving disease inactivity in the majority of the patients, improving BCVA and decreasing the dose of corticosteroids. Adverse events and side effects are limited. Pre-screening with MRI of the brain is recommended in paediatric patients with intermediate and panuveitis.

## INTRODUCTION

Paediatric uveitis is a severe inflammatory eye disease, which can lead to visual loss in one-third of the patients, and eventually to permanent blindness.[1] The pathophysiology of non-infectious uveitis still remains mostly unknown. Uveitis in childhood can be related to systemic conditions of which juvenile idiopathic arthritis (JIA) is by far the most common and well-studied.[2–4] It is considered to be a multifactorial autoimmune disorder with various (epi)genetic predisposing factors and involvement of pro-inflammatory cytokines such as tumour necrosis factor α (TNFα).[5,6]



Goals of treatment in paediatric uveitis are to preserve vision and prevent ocular complications, by controlling the inflammation and achieving a stable remission as early as possible.[7] Corticosteroids are traditionally used as first-line treatment. However, they can lead to severe ocular side effects such as cataract and glaucoma, and have potential severe risks for the general child's health.[7,8] Therefore, switching to systemic immunomodulatory therapy (IMT) in the early course of uveitis is state of the art nowadays.[7,8]

Adalimumab, a recombinant human anti-TNFα monoclonal antibody, has shown to be an effective and safe therapeutic in JIA-uveitis regarding the control of inflammation, improvement of visual acuity, and reducing glucocorticoid use.[7,9,10] Despite the efficacy of adalimumab in treating uveitis, precautions are needed, since there is upcoming evidence suggesting an association between TNFα blocking agents and demyelination of the nervous system.[11–14] Intermediate uveitis has been in 7-10% associated with multiple sclerosis, but also patients with a history of optic neuritis or papillitis could be at increased risk.[15] Although less common than in intermediate uveitis, children with other types of uveitis may have pre-existent and asymptomatic white matter abnormalities and/or demyelination as well, which could potentially progress under anti-TNFα treatment.[12]

Studies involving clinical efficacy of adalimumab in non-JIA paediatric uveitis are scarce.[16–18] Currently, adalimumab is used off-label in Europe in paediatric patients with intermediate, posterior and panuveitis. Therefore, the objective of this study is to investigate retrospectively the efficacy and potential risks of treatment with adalimumab in chronic paediatric non-JIA-uveitis.

# METHODS

## Patient identification

The medical records from the department of ophthalmology, University Medical Centre Utrecht, the Netherlands, were reviewed to identify all patients with paediatric uveitis. Patients and/or their parents/caretakers were asked to give informed consent to review their medical records. The study adhered to the tenets of the Declaration of Helsinki and was conducted after ethical approval for data collection by the Medical Ethical Committee Utrecht (protocol number: 18-751/C).

Patients eligible for treatment with adalimumab were diagnosed with refractory non-JIA-uveitis and/or if the necessary dosage of IMT and/or oral corticosteroids was leading to complications. Uveitis was defined as refractory when patients did not achieve a 2-step decrease in the level of inflammation in the anterior chamber and/or vitreous humour despite the use of systemic IMT and/or corticosteroids within at least six months. The reasons for starting adalimumab did not change significantly over the period of the study.

Before starting the additional treatment with adalimumab, to exclude associated systemic disease, children were screened with magnetic resonance imaging (MRI) of the brain or where indicated with visual evoked potential (VEP) to exclude the risk of demyelinating disease.[13] The children were also screened for tuberculosis and hepatitis.

Inclusion criteria were: treatment with adalimumab (Humira®; AbbVie Inc., Ludwigshafen, Germany), diagnosis of non-JIA-uveitis, onset of uveitis before 16 years of age, and a minimum follow-up of six months. The patients had been treated between January 2008 and March 2020. Diagnosis and treatment of uveitis was performed by an ophthalmologist specialized in paediatric uveitis and a paediatric immunologist according to the National Guidelines Uveitis. The diagnostic criteria for uveitis were defined as according to the Standardization of Uveitis Nomenclature (SUN) criteria.[19]

Participants received adalimumab at a dose of 20 mg in patients weighing < 30 kg, or 40 mg in patients weighing ≥ 30 kg, administered as a subcutaneous injection every two weeks. The dose and the interval could in some cases be adjusted according to the level of disease activity.

## Data collection

Data were retrieved retrospectively from visits at our outpatient ophthalmology clinic at standard time intervals: before start of adalimumab (baseline), 3, 6, 9, 12 months, and then yearly after starting adalimumab.

Data extracted from the electronic patient files included: demographics, uveitis characteristics, underlying systemic disease, date of adalimumab start, use of other medication, side effects, and complications. The following side effects and complications were registered: cataract requiring surgery, secondary glaucoma, cystoid macular oedema (CME), papillitis, strabismus, amblyopia, and abnormal laboratory results (liver enzymes, kidney function, haemoglobin, and leucocytes). Secondary glaucoma was defined as glaucomatous visual field defect in combination with intraocular pressure (IOP) higher than 21 mmHg, and glaucomatous cupping of the optic nerve or progressive thinning of the ganglion cell layer and retinal nerve fibre layer (RNFL) on optical coherence tomography (OCT).[20] CME was defined as the presence of macular thickening with cyst formation observed by OCT or late phase leakage on fluorescein angiography (FA) scored according to the Angiography Scoring for Uveitis Working Group (ASUWOG).[21] Papillitis was defined as blurring of the optic disc margins and/or as optic disc hyperfluorescence on FA scored according to the ASUWOG criteria. Anti-adalimumab antibodies were measured on indication, when patients did not achieve a 2-step decrease in the level of inflammation as according to the SUN criteria, or when the physician had doubts about the therapy compliance of the patient.[22] This policy did not change significantly over the period of the study.

## Main outcome measures

The primary endpoint was the proportion of patients with disease inactivity or improvement from baseline for a minimum period of three months and with no development or worsening of any new ocular complications. Disease inactivity and improvement were defined according to the SUN criteria.[22] Disease inactivity was defined as anterior chamber cell count of <1+ cells in anterior uveitis and panuveitis, and <1+ cells in the vitreous humour in intermediate, posterior and panuveitis in absence of papillitis, CME and/or vasculitis. Disease improvement was defined as two grade decrease in the anterior chamber and/or vitreous cavity.

Secondary endpoints were: time to inactivity of uveitis, improvement of visual acuity with two Snellen lines, taper of topical or systemic corticosteroids, time to first uveitis relapse on adalimumab therapy (excluding activity of uveitis up to three months after an eye surgery), improvement of CME on FA and/or OCT by 20%, and total decrease of FA-score with ≥25%.[21]

### Statistical analysis

Descriptive statistics were used to report demographic and clinical features of the patients. McNemar test was used to analyse linked dichotomous variables. Wilcoxon test for paired samples was used to analyse means of abnormally distributed linked samples. Cox proportional hazard (PH) regression was used to identify factors associated with outcomes. The PH assumption was verified visually by examining the hazard plots and statistically by including the interaction between the logarithm of time and the covariate of interest in a univariate model. The PH assumption was considered suspect if the interaction between time and covariate of interest was significant at the 0.1 level. Variables that changed over time were evaluated as time-updated variables. Correction for analysis of paired eyes was performed using generalized estimating equations (GEE). *P*-values of less than 0.05 were considered statistically significant. All significances are 2-tailed. All statistical analyses were performed with SPSS 25 (SPSS Inc., Chicago, Illinois, USA).

## RESULTS

Fifty children with non-JIA-uveitis were eligible for treatment with adalimumab. Twenty-six children were screened before treatment with MRI of the brain, of whom seven (27%) children were diagnosed with anterior uveitis, seven (27%) with intermediate uveitis and twelve (46%) with panuveitis. Adalimumab was relatively contraindicated in six of them (23%) based on pre-existing white matter abnormalities of the brain, and in one patient based on an abnormal VEP (2%) (**Figure 4.1**). Five patients with pre-existing white matter abnormalities of the brain had panuveitis and one patient had intermediate uveitis. Papillitis was seen in five of the six patients with abnormities on MRI. For subsequent analyses, we included 43 children (81 affected eyes) with non-JIA-uveitis who started additional treatment with adalimumab, and had a minimum follow-up of six months.

### Characteristics of children before treatment of adalimumab

**Table 4.1** summarizes the general patient characteristics before additional treatment with adalimumab (baseline). Fifteen (35%) of the patients treated with adalimumab, were diagnosed with anterior uveitis and 28 children (65%) with non-anterior uveitis. Median age at baseline was eleven years (range 4-17 years). Median duration of uveitis at baseline was two years (range 0-9 years). Topical corticosteroids were used in 91% of the children ($n$ = 70 affected eyes of 39 children), of whom 44% ($n$ = 17) used topical corticosteroids > 3 drops daily. Thirty-one children (72%) were treated with systemic corticosteroids. Forty-one children had active uveitis at baseline. In the

other two patients, adalimumab was initiated to reduce the high dosage of topical and systemic corticosteroids which was needed to maintain disease inactivity.

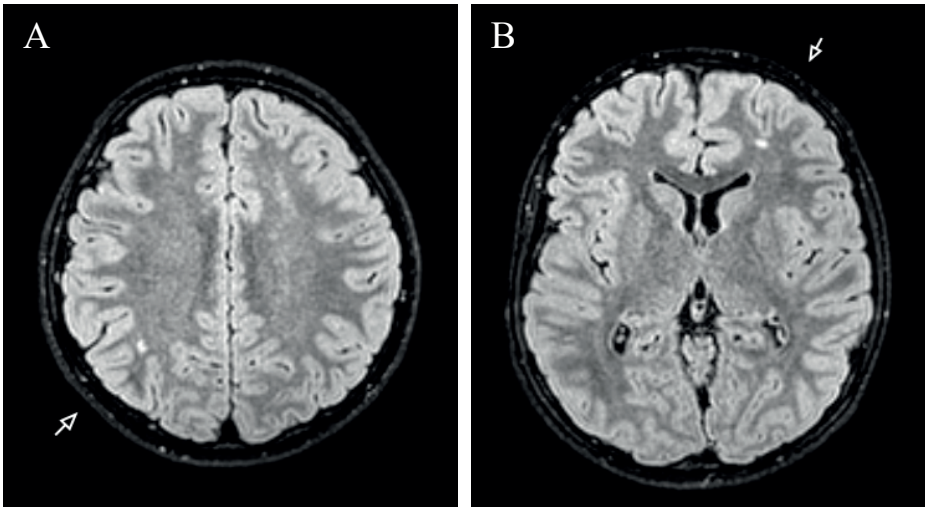


**Figure 4.1** The brain MRI of a 13-year-old boy with pediatric panuveitis is shown. **A**, Axial FLAIR MRI with the arrows showing white matter abnormalities localized right parietal, and **B**, left frontal. These focal white matter abnormalities could be based on small focal areas of demyelination. Based on these white matter abnormalities the patients deemed ineligible for treatment with adalimumab.

**Table 4.2** shows the clinical disease activity at baseline. Active inflammation in the anterior chamber was present in 27% (*n* = 22) of the affected eyes, in the vitreous humour in 19% (*n* = 15) of the affected eyes, and both in the anterior chamber and the vitreous humour in 26% (*n* = 21) of the affected eyes. Twenty-eight percent of the affected eyes (*n* = 23) had no active inflammation in both the anterior chamber and the vitreous humour at baseline.

Ocular secondary complications of uveitis present before adalimumab treatment included: cataract requiring surgery (*n* = 17), papillitis (*n* = 13), ocular hypertension (*n* = 13), cystoid macular oedema (*n* = 11), glaucoma (*n* = 4), and amblyopia (*n* = 1).

**Table 4.1** General baseline characteristics of children with idiopathic uveitis at the time adalimumab was started.

| **Pre-screening** | **Data, *n* = 50** |
|---|---|
| White matter abnormalities on MRI of the brain, *n* (%) | 6/26 (23.1) |
| Abnormal VEP, *n* | 1 |
| Tuberculosis, *n* | NA |
| Hepatitis, *n* | NA |
| **Demographics** | **Data, *n* = 43** |
| Bilateral disease, *n* (%) | 38 (88.4) |
| Female gender, *n* (%) | 29 (67.4) |
| Anatomic type of uveitis, *n* (%) | |
| Anterior uveitis | 15 (34.9) |
| Intermediate uveitis | 8 (18.6) |
| Posterior uveitis | NA |
| Panuveitis | 20 (46.5) |
| Systemic diagnosis, *n* (%) | |
| Presumed TINU syndrome | 4 (9.3) |
| Blau-syndrome | 3 (6.7) |
| Other (Hashimoto, VKH disease) | 2 (4.7) |
| Age in years | |
| At onset of uveitis, median (range) | 8.0 (3-15) |
| At start of adalimumab, median (range) | 11.0 (4-17) |
| Duration of uveitis at start of adalimumab, median (range) | 2.0 (0-9) |
| **Medication** | |
| Topical steroids > 3 drops daily, *n* (%) | 17 (39.5) |
| Systemic corticosteroids, *n* (%) | 31 (72.1) |
| IMT, *n* (%) | |
| Non | 1 (2.3) |
| MTX | 21 (48.8) |
| MMF | 17 (39.5) |
| Mycophenolic acid | 2 (4.7) |
| MTX + MMF | 2 (4.7) |

IMT = Immunomodulatory therapy; MRI = magnetic resonance imaging; MTX = methotrexate; MMF = mycophenolate mofetil; NA = not applicable; TINU = Tubulointerstitial nephritis and uveitis; VEP = visual evoked potential; VKH = Vogt-Koyanaga-Harada.

**Table 4.2** Ocular characteristics before treatment of adalimumab (baseline), after 6, and 24 months of follow-up, *n* = 81 eyes.

| Ocular characteristics | Baseline | 6 months | *P*-values | 24 months | *P*-values |
|---|---|---|---|---|---|
| Best corrected visual acuity (logMAR score) Mean (SD)[a] | 0.16 (0.55) | 0.08 (0.24) | 0.092 | 0.05 (0.19) | **0.015** |
| Clinical disease activity, *n/N* (%) | | | | | |
| Anterior chamber cells ≥1+ [b] | 44/81 (54.3) | 7/79 (8.9) | **<0.001** | 10/58 (17.2) | **<0.001** |
| Flare score ≥1+ [b] | 14/22 (63.6) | 9/30 (30.0) | 1.000 | 5/29 (17.2) | 0.250 |
| Vitreous haze score ≥ 1+ [b] | 11/24 (45.8) | 2/14 (14.3) | 0.688 | 3/21 (14.3) | 1.000 |
| Vitreous humour cells ≥1+ [b] | 36/63 (57.1) | 14/60 (23.3) | **0.001** | 6/46 (13.0) | **<0.001** |
| Cystoid macular oedema (active inflammation) [b] | 15/81 (18.5) | 6/79 (7.6) | **0.004** | 2/58 (3.4) | **0.013** |
| Papillitis [b] | 24/81 (29.6) | 12/79 (15.2) | **0.001** | 2/58 (3.4) | **0.001** |
| Fluorescein angiography score, median (range) [a] | 13.5 (1-23) | 5.0 (0-15) | 0.258 | 2.0 (0-19) | **0.001** |
| Newly diagnosed Comorbidities/ Complications, *n/N* (%) | | | | | |
| Amblyopia | 2/81 (2.5) | NA | | NA | |
| Glaucoma | 5/81 (6.2) | NA | | 1/53 (1.9) | |
| Ocular hypertension | 19/81 (23.5) | NA | | NA | |
| Cataract surgery | 24/81 (29.6) | 2/56 (3.6) | | NA | |
| Topical steroid drops, | | | | | |
| Eyes on > 3 drops topical steroids daily, *n/N* (%)* [a] | 27/81 (33.3) | 14/75 (18.7) | **0.004** | 4/58 (6.9) | **<0.001** |
| Dosage of topical steroids daily, median drops (range) [a] | 2 (0-6.5) | 1 (0-4.5) | **<0.001** | 0.6 (0-4.5) | **<0.001** |

NA = not applicable; SD = standard deviation

Boldface indicate that P < 0.05. All p-values are compared with the moment of start of adalimumab.

Anterior chamber activity, flare score, vitreous cell activity, and vitreous haze is scored according to the recommendations of SUN working group.22

[a] P-values computed with Wilcoxon Signed Ranks Test.

[b] P-values computed with McNemar Test.

## Primary and secondary outcomes

Median duration of study follow-up was 2.5 years (range 0.5-11.3 years). Disease inactivity of uveitis was achieved in 91% of the patients after a median of three months (range 3-33) of treatment, *P* < 0.001 (**Figure 4.2**). The median dosage of systemic corticosteroids reduced from 10 mg/day at baseline (range 0-60 mg/day), to 5 mg/day at three months (range 0-20 mg/day) (*P* < 0.001), and to 0 mg/day at 24 months (range 0-15 mg/day) (*P* < 0.001) (**Figure 4.2**). Best corrected visual acuity (BCVA) improved from 0.16 ± 0.55 logarithm of the minimum angle of resolution (logMAR) at baseline to 0.08 ± 0.26 logMAR at 9 months (*P* <0.001), and to 0.05 ± 0.019 logMAR at 24 months (*P* < 0.05) of follow-up (**Table 4.2**). Within the first year of adalimumab use, 35% of the patients (*n* = 15) had an improvement of visual acuity with two Snellen lines in at least one eye.

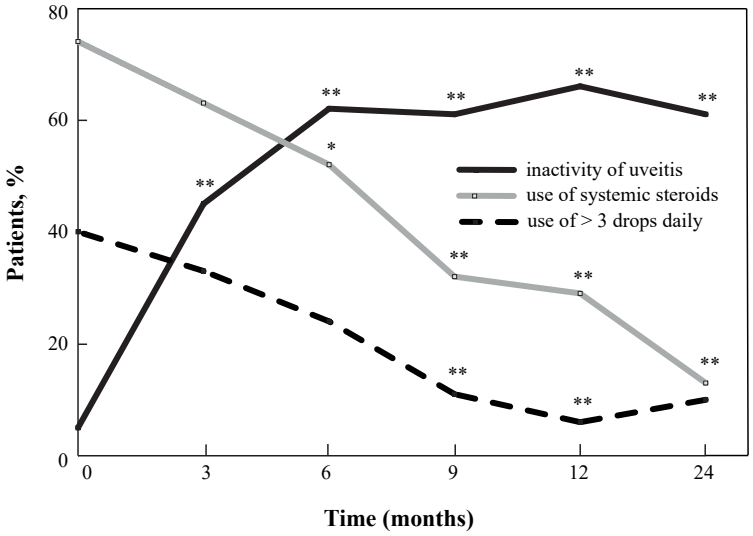


**Figure 4.2** Percentage of patients with inactivity of uveitis, the use of systemic corticosteroids, and the use of topical corticosteroids dosed at more than three drops daily during the treatment with adalimumab. N = 43 children with idiopathic paediatric uveitis. *P*-values computed with McNemar Test. * $P < 0.05$, ** $P < 0.01$.

An OCT-scan was performed in 63 of the 81 affected eyes (78%) before the start of adalimumab. Fifteen of the scanned eyes had CME with an increased CMT on OCT. An improvement of ≥20% CMT reduction on OCT occurred in 53% of the eyes with CME (*n* = 8). Six eyes showed this reduction in the first three months. Thirty-one patients (72%) had undergone a FA in the first year after the start of adalimumab therapy, of which eight children had undergone a FA the second year. In seven of these patients (88%), an improvement of ≥25% of the FA score in the second year of follow-up was achieved. After 24 months the median FA score was improved significantly to 2.0 compared to baseline 13.5 ($P < 0.001$).

## Relapse of uveitis

Relapse of uveitis occurred in 19 (49%) of the 39 patients who achieved disease inactivity, after a median of 14 months (range 3-40 months). In 13 patients (68%) a relapse occurred within 12 months of disease remission, and in six patients (32%) after 12 months. Fourteen patients (74%) had an identifiable cause of relapse: dose reduction or discontinuation of medication (*n* = 7), lack of therapy compliance (*n* = 6), or anti-adalimumab antibodies (*n* = 1). In the group without an identifiable cause of relapse, the median duration of adalimumab controlled remission until relapse was 5.5 months (range 3-16 months).

## Comorbidities and complications

Comorbidities and complications whether or not related to treatment with adalimumab during follow-up were infections (*n* = 3): scarlet fever, pharyngitis, and influenza, causing a short interruption in the use of adalimumab for a maximum of two doses, not leading to hospitalization. During follow-up no laboratory abnormalities which could be related to adalimumab were identified. Seven patients (16%) had elevated liver enzymes up to two times the normal value. In all six patients (14%) who underwent cataract surgery during adalimumab therapy, the cataract was already present before adalimumab treatment. Two patients were diagnosed with secondary glaucoma.



Adalimumab was discontinued in thirteen children (30%) due to ineffectiveness (*n* = 8, after 8-105 months, of whom one patient had positive anti-adalimumab antibodies), long-term disease inactivity (*n* = 3, after 31-44 months), side-effects (urge-incontinence) (*n* = 1, after 4 months) or own initiative (*n* = 1, after 75 months). The dose of adalimumab was adjusted to weekly administration in eleven children (26%), of whom six discontinued adalimumab due to ineffectiveness (*n* = 5) or own initiative (*n* = 1). Relapse of uveitis occurred after a median of eight months (range 7-9 months) in all three patients who discontinued adalimumab due to long-term disease inactivity. Two of them used methotrexate, with one child also using topical corticosteroids. One child used no additional medication.

## Regression analysis

Univariate analysis of predictive factors for inactivity of uveitis during adalimumab treatment showed that boys achieved disease inactivity faster with a hazard ratio (HR) of 3.34 (95% CI 1.54-7.27, *P* = 0.002). This was not the case for the anatomic classification of uveitis, age, and type of IMT used. Multivariate analysis confirms these results, with male gender being independently associated with a faster achievement of disease inactivity (HR 3.12, 95% CI 1.39-6.99, *P* = 0.006) (**Figure 4.3**; **Supplemental information).**

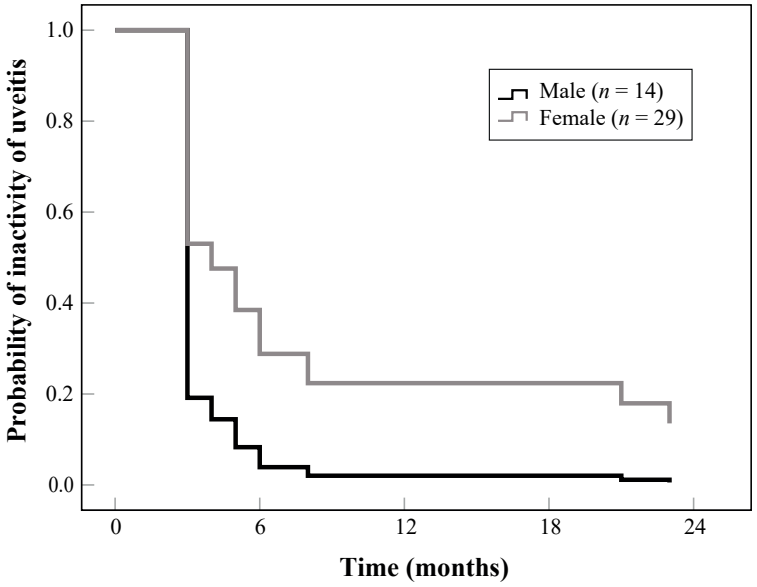


**Figure 4.3** Graph showing the probability of disease inactivity of uveitis as a function of gender after initiation of treatment with adalimumab. N = 43 children with idiopathic paediatric uveitis. *P*-value < 0.01.

## DISCUSSION

This retrospective cohort study focusses on the additional treatment with adalimumab used off-label in paediatric uveitis not associated with JIA. We demonstrate that treatment with adalimumab led to prompt inactivity of uveitis in the vast majority of the children (91%). Until now the effectiveness of adalimumab was only shown for paediatric uveitis associated with JIA (SYCAMORE trial), adults with JIA-uveitis (ADJUVITE trial), or for non-infectious uveitis in adults (VISUAL III trial). These trials showed a treatment response of 56-73% in the participants of the adalimumab group.[10,14,23] Two systematic reviews reported a pooled response rate of 87% (95% CI 75-98%) in chronic paediatric uveitis with mostly JIA.[24,25]

Although these studies include patients with different types of uveitis or adults, the results are in accordance with our findings. We also found that 20 patients achieved inactivity without the occurrence of a relapse during the variable follow-up. This suggests that within 6 to 24 months of follow-up 50% of those achieving an early response will maintain a persistent state of inactivity. Long-term follow-up of these patients is rarely reported, but the rates of steroid reduction over two years in this study suggests that weaning of treatment in a significant number of patients is possible.

A growing number of studies describe central and peripheral nervous system demyelinating events during the use of TNFα inhibitors, suggesting a possible relation between the use of anti-TNFα agents and demyelination.[11,13,14,26,27] The literature regarding pre-screening with brain MRI is ambiguous as there are no clear guidelines.[13,28] We performed our pre-screening with MRI of the brain in children without an underlying systemic disease and detected white matter abnormalities in twenty-three percent of them. Although, no diagnosis of MS or other central nervous system condition could be made, we did not take the risk of treating these patients with adalimumab as nowadays other therapeutic options for refractory uveitis without this risk of demyelination emerge, like IL-6 inhibitors (i.e. tocilizumab).[29] Interestingly, only one of our patients with abnormalities on MRI had intermediate uveitis, which is the most reported presentation of uveitis in relation to multiple sclerosis and white matter abnormalities on brain MRI.[12,15] The other five children were diagnosed with panuveitis, which emphasizes the need for the awareness of this potential risk by the treating physicians also in other forms of uveitis. Remarkable is that five of the six patients with abnormities on MRI were diagnosed with papillitis. This possible association should be investigated in future research. Initially normal brain MRI was repeated in four patients during adalimumab treatment and showed no signs of demyelination. Also no clinical signs of demyelinating events were observed during treatment. Based on our results, we recommend screening with MRI of the brain before the start of adalimumab in case of intermediate or panuveitis.

To preserve vision in paediatric uveitis, it is crucial to achieve disease inactivity as fast as possible with limited use of topical and systemic corticosteroids, and to prevent development of secondary vision threatening complications of uveitis. One of these complications is cataract, which is increasing more rapidly when topical corticosteroids are dosed at > 3 drops daily.[30] In our study, we observed a relatively quick reduction in the use of topical corticosteroids during adalimumab treatment, reaching statistical significance after nine months of therapy. Equally important, we observed an impressive reduction of systemic corticosteroids during adalimumab treatment that reached statistical significance after six months. Chronic use of systemic corticosteroids involves serious health care risks in paediatric patients, such as ocular hypertension, Cushing's syndrome, hyperglycaemia, osteoporosis, and growth retardation.[7,8] Prior to the use of adalimumab, at least three children had clinical signs of Cushing's syndrome, and one child developed osteoporosis due to long-term use of systemic corticosteroids. Therefore, it is essential that the (long-term) use of systemic corticosteroids is minimized.

Although a good effectivity of adalimumab in reaching remission is being shown in our and other studies, a relapse of uveitis still occurs in a significant number of the patients.[9,10,14,16,31] In our study, uveitis flared-up in five of the children (13%) without an identifiable cause during adalimumab treatment and in 14 (33%) with an identifiable cause. Drug immunogenicity is one of the few described risk factors linked to failure of anti-TNFα treatment in non-infectious uveitis.[32] Hence, anti-adalimumab antibodies could play a role in the occurrence of uveitis relapse. In our study, three patients had developed anti-adalimumab antibodies resulting in discontinuation of the drug in one patient. Two of these patents had chronic anterior uveitis (one had ANA positive serology), and one was diagnosed with panuveitis (ANA negative). Antibodies were found in 16-26% of the patients in studies with JIA patients. Results of a Finnish study shows that anti-adalimumab antibody levels of ≥ 12 AU/ml were associated with a higher grating of activity of uveitis, a higher failure to reach disease remission and a lack of concomitant methotrexate therapy in JIA-associated patients.[33–35] Unfortunately, data regarding non-JIA-uveitis are unknown. Our clinical experience is that after increasing the frequency from every other week to weekly, the antibodies can disappear and a control of inflammation can be attained. In some cases it is possible to set the frequency later on back to every other week. In practice, when regarding treatment failure or its in-effectivity, serum anti-adalimumab antibodies can be analysed together with the serum blood level of adalimumab.

Our study found a positive association between male gender and time to achieve disease inactivity of uveitis. To our knowledge this positive finding is not described in literature before, while there is upcoming evidence that there are gender differences in uveitis in children for example in the risk of uveitis and in the severity of the inflammation.[36–39]

Recent studies show that increasing adalimumab administration to weekly in patients with inadequate inflammatory control on every other week, is a reasonable treatment option.[40–42] We do suggest to consider weekly administration of adalimumab when there is no improvement in disease activity or in the case of presence of anti-adalimumab antibodies. An increase to weekly administration took place in one-fourth of our patients. Disease inactivity was reached in fifty percent of these children with weekly administration of adalimumab. Treatment of these anti-TNFα resistant patients can be challenging. Recent literature suggests switching to: another type of anti-TNFα, tocilizumab, or other more experimental agents in paediatric uveitis such as abatacept, rituximab or a janus kinase inhibitor.[29,43,44]

Seven patients (16%) had elevated liver enzymes up to two times the normal value. In all of them, the elevated liver enzymes normalized after dose adjustment of the conventional IMT. After dose adjustment only one patient had a relapse of uveitis. As a response the dose of the conventional IMT was increased and the disease activity improved. The dose of adalimumab was not adjusted in these patients. Therefore, our opinion is that these elevated liver enzymes are due to the use of the conventional IMT. However, we cannot exclude the role of adalimumab completely, but we do not think it was significant.



No major side effects or adverse events occurred in our study, resulting in the safe use of adalimumab in the home situation in contrast to other biological agents such as infliximab, which can only be administered intravenously in the hospital. The multidisciplinary team of ophthalmologists and paediatricians needs to make a trade-off between potential vision loss and potential risk of steroid-related complications on one hand and the risk of infection during adalimumab treatment on the other hand. This emphasizes the importance of a multidisciplinary approach in the treatment of paediatric uveitis by an ophthalmologist and a paediatric immunologist.

The study is limited by its retrospective design. Nonetheless, all data were structurally noted in the electronic patient files with a low inter-observer variability between two paediatric uveitis specialists, using the same protocolled treatment strategies and a uniform way of registration in the expert centre for paediatric uveitis in the Netherlands.

## CONCLUSIONS

This study shows the effectiveness of anti-TNFα therapy with adalimumab treatment of chronic paediatric non-JIA-uveitis. Adalimumab therapy leads to improvement of disease inactivity, BCVA, and decrease in the use of corticosteroids. We recommend MRI of the brain as a screening before starting adalimumab in intermediate and panuveitis.

## ACKNOWLEDGEMENT

This work was financially supported by AbbVie B.V., Hoofddorp, The Netherlands, (grant no.: SA-002407). The sponsor had no role in the design or conduct of this research.

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# SUPPLEMENTARY DATA

**Supplemental Table 4.1** Univariate analysis of the impact of patient characteristics on inactivity of uveitis

| Characteristics at baseline | Inactivity of uveitis | | |
|---|---|---|---|
| | HR | 95% CI | *P*-values |
| Male gender | 3.34 | 1.54-7.27 | **0.002** |
| Anatomic classification of uveitis | | | |
| Anterior uveitis | 1.00 | | |
| Intermediate uveitis | 0.61 | 0.21-1.72 | 0.345 |
| Panuveitis uveitis | 0.92 | 0.45-1.88 | 0.823 |
| Age | 0.97 | 0.86-1.08 | 0.558 |
| MTX treatment | 0.74 | 0.39-1.41 | 0.362 |

CI = confidence interval; HR = hazard ratio; MTX = methotrexate.
Boldface indicate that $P < 0.05$.

**Supplemental Table 4.2** Multivariate analysis of the impact of patient characteristics on inactivity of uveitis

| Characteristics at baseline | Inactivity of uveitis | | |
|---|---|---|---|
| | HR | 95% CI | *P*-values |
| Male gender | 3.12 | 1.39-6.99 | **0.006** |
| Anatomic classification of uveitis | | | |
| Anterior uveitis | 1.00 | | |
| Intermediate uveitis | 0.47 | 0.15-1.44 | 0.184 |
| Panuveitis uveitis | 0.76 | 0.36-1.61 | 0.469 |
| Age | 0.95 | 0.84-1.06 | 0.347 |
| MTX treatment | 0.76 | 0.37-1.57 | 0.465 |

CI = confidence interval; HR = hazard ratio; MTX = methotrexate.
Boldface indicate that $P < 0.05$.

# CHAPTER 5

## Serum biomarkers of vascular involvement in childhood uveitis

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*Adapted version accepted for publication in Translational Vision Science and Technology*

# ABSTRACT

## Purpose

Nonanterior uveitis frequently involves the retinal vasculature. However, no molecular markers associated with the retinal vascular disease are currently known. In this study, we aimed to identify serum biomarker signatures associated with retinal vascular involvement in noninfectious pediatric uveitis.

## Methods

We performed a 384-plex targeted proteomic analysis of serum samples of 154 noninfectious pediatric uveitis patients diagnosed with nonanterior uveitis (*n* = 74), idiopathic chronic anterior uveitis (iCAU, *n* = 36), juvenile idiopathic arthritis associated uveitis (JIA-U, *n* = 44), as well as of 22 noninflammatory pediatric controls. Data on retinal vascular involvement (i.e., papillitis, cystoid macular edema, retinal vasculitis, or retinal capillary leakage on optical coherence tomography and/or fluorescein angiography) were used to stratify cases in the nonanterior uveitis group.

## Results

In analysis of nonanterior uveitis, we identified 9 proteins significantly associated with retinal vascular involvement, including F13B, MYOM3, and PTPN9. These proteins were enriched through pathway enrichment analysis for the coagulation cascade. Comparing cases and controls, we identified 63 differentially expressed proteins, notably proteins involved in platelet biology and complement cascades, which could be primarily attributed to differences in serum proteomes between anterior uveitis and nonanterior uveitis groups.

## Conclusions

Serum proteins related to the coagulation and complement cascade are associated with retinal vascular involvement in pediatric uveitis patients. Our results indicate involvement of mediators that could interact with the microcirculation in pediatric uveitis and might serve as potential biomarkers in personalized medicine in the future.

# INTRODUCTION

Noninfectious pediatric uveitis is a complex inflammatory ocular condition, accounting for 5-10% of all uveitis cases.[1] Pediatric uveitis can occur in relation to autoimmune diseases such as juvenile idiopathic arthritis (JIA); however, most uveitis in children is idiopathic.[2] Children frequently have a severe chronic disease course with a high potential risk of developing vision-threatening complications. There is a higher risk of vision loss among pediatric uveitis patients with retinal vascular involvement, which accounts for 40% of all pediatric uveitis cases.[2] Retinal vascular involvement includes cystoid macular edema (CME), retinal vasculitis, and retinal capillary leakage, which require lengthy treatment or result in treatment failure and poor visual outcome.[3,4] Early identification of patients at risk of posterior involvement may enable early treatment to prevent vision-threatening complications. Retinal vascular involvement is monitored by clinical assessment through imaging. Molecular mechanisms that predispose cases to posterior involvement remain poorly understood and currently no biomarkers are known.



The rapid development of blood proteomic technologies provides new insights into the pathophysiological mechanisms, discovering biomarkers and putative targets for treatment. The simultaneous quantification of hundreds of proteins using multiplexed immunoassays has successfully aid in our understanding of inflammatory conditions, including uveitis.[5–7] Application of targeted proteomics may help to identify the pathways associated with retinal vascular involvement in pediatric uveitis. In this study, we aimed to identify molecular signatures associated with the involvement of the retinal microcirculation through targeted proteomics of serum of patients with noninfectious pediatric uveitis.

# METHODS

## Patients and sample collection

This study adhered to the tenets of the Declaration of Helsinki and was conducted in compliance with the Medical Ethics Research Committee of the University Medical Center Utrecht (protocol number: 22-1036). Written informed consent was obtained from all participants and/or representatives after explanation of the nature and possible consequences of the study. Serum blood samples were collected from 154 children with noninfectious pediatric uveitis and 22 pediatric controls without (a history of) inflammatory disease during surgery indicated for strabismus. Patients with pediatric uveitis consisted of cases with nonanterior uveitis (intermediate and

panuveitis combined, *n* = 74), idiopathic chronic anterior uveitis (iCAU, *n* = 36), and juvenile idiopathic arthritis associated uveitis (JIA-U, *n* = 44). Serum was collected at the outpatient department of the University Medical Center Utrecht (UMCU), The Netherlands, (uveitis biobank, *n* = 158), or at the diagnostic laboratory at the UMCU (the remainder of samples obtained for diagnostic purposes, *n* = 18) between January 2013 and January 2023. The children were aged <18 years at time of sampling. The initial diagnosis of uveitis was established by a trained uveitis specialist in accordance to the *Standardization of Uveitis Nomenclature* (SUN) criteria.[8] All patients were referred to a pediatric rheumatologist for the presence of underlying systemic disease. The diagnosis of systemic diseases was in concordance with the current diagnostic criteria. For each sample, we collected age, sex, anatomic subtype of uveitis, presence of an associated systemic condition, uveitis activity, and laboratory results for antinuclear antibody (ANA) testing, as well as the use of systemic treatment with corticosteroids, immunomodulatory therapy (IMT), and/or biological agents.

## Assessment of retinal vascular involvement

The presence of retinal vascular involvement was determined for patients with non-anterior uveitis based on the presence of any of the following features in one or both eyes:

1) retinal vasculitis on fluorescence angiography (FA);[11]
2) diffuse capillary leakage on FA;[11]
3) papillitis defined as the presence of hyperfluorescence and/or leakage of the optic disc on FA[4] and/or average retinal nerve fiber layer thickness > 130 µm on optical coherence tomography (OCT) with the exclusion of all other noninflammatory causes of optic disc swelling;[12]
4) cystoid macular edema (CME) defined as the presence of macular thickening with cyst formation visible on macular OCT and/or FA.[13–15]

FA was performed within 6 months before or after sampling and OCT within 3 months before or after sampling.

## Serum Proteomic Olink Analysis

Serum tubes were kept for 30 minutes at room temperature, centrifuged at 2000g for 10 minutes at room temperature, and stored directly at −80° C until assayed. Targeted proteomics of the serum samples was performed in close collaboration with Olink using their proximity extension immunoassay (PEA; Olink Proteomics, Uppsala Sweden) with the Explore 384 inflammation II panel measured by next-generation sequencing.[16,17] The obtained protein concentrations are expressed as *Normalized*

*Protein eXpression* (NPX) values, an arbitrary unit or procedure defined unit on a log2 scale of the serum concentration of each protein.

Five proteins did not meet Olink's batch release quality control criteria and are therefore not included in the analysis (**Supplementary Figure S5.1**). Based on protein detection information returned by Olink, 323 out of the 365 proteins (88%) were detected in >55% of the samples. The full list of protein targets (*n* = 365) is provided in **Supplementary Table S5.1**.

### Statistical analysis

All statistical analysis were performed in *R* version 4.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Preprocessing of the Olink data output was conducted using the *OlinkAnalyze* R package[18] using the *Olink_qc_plot* and *Olink_dist_plot* functions. Principal component analysis was conducted using the *factoextra* package.[19] Differential expression analysis was conducted using a Likelihood Ratio function adjusting for age and sex. The *qvalue* R package was used for false discovery rate (FDR) estimation and q-value < 0.05 was considered statistically significant. We made the following group comparisons; (1) pediatric controls versus nonanterior uveitis, iCAU and JIA-U patients, (2) anterior versus nonanterior uveitis, and (3) nonanterior uveitis patients with and without retinal vascular involvement. Pathway enrichment analysis was conducted using the *clusterProfiler* R package[20] using Reactome as a reference database.[21]

## RESULTS

In total, 176 participants were included in the study: nonanterior uveitis (*n* = 74), iCAU (*n* = 36), JIA-U (*n* = 44), and controls (*n* = 22). The nonanterior uveitis patients consisted of 21 patients diagnosed with intermediate uveitis and 53 patients with panuveitis. Demographic characteristics of the participants are shown in **Table 5.1**.

**Table 5.1** Demographic characteristics

| Characteristic | Pediatric control | Nonanterior uveitis | iCAU | JIA-U | *P*-value |
|---|---|---|---|---|---|
| N | 22 | 74 | 36 | 44 | |
| Male, *n* (%) | 8 (36) | 32 (43) | 11 (31) | 15 (34) | 0.578 |
| Age at sampling in years, median (IQR) | 8 (5-11) | 13 (10-14) | 11 (8-13) | 10 (7-12) | **<0.001** |
| Active uveitis, *n* (%) | NA | 53 (72) | 11 (31) | 24 (55) | **<0.001** |
| ANA seropositivity, *n* (%) | NA | 17/69 (25) | 21/36 (58) | 34/41 (82) | **<0.001** |
| Systemic therapy | | | | | |
| None | 22 (100) | 31 (42) | 14 (39) | 13 (30) | |
| Corticosteroids | 0 | 13 (18) | 2 (6) | 2 (5) | |
| csDMARD | 0 | 38 (81) | 21 (58) | 30 (68) | |
| Biological agent | 0 | 10 (14) | 4 (11) | 0 | |

ANA = Antinuclear Antibodies, csDMARD = synthetic disease-modifying antirheumatic drug, iCAU = idiopathic Chronic Anterior Uveitis, IQR = Interquartile Range, JIA-U = Juvenile Idiopathic Arthritis associated Uveitis, NA = Not Applicable.

## Group comparisons

We conducted serum proteomic analysis of 154 patients and 22 controls adjusted for age and sex. The serum proteome of patients and controls showed 63 differentially expressed proteins (q<0.05), of which Pigment epithelium-derived factor (SERPINF1, Serpin Family F Member 1, *q*=5.4E-5) a potent inhibitor of angiogenesis, Afamin (AFM, *q*=1.0E-4) involved in vitamin E binding, Complement Factor H related 2 (CFHR2, *q*=1.4E-4) and Kallistatin (SERPINA4, *q*=0.001) were the most differentially expressed (**Figure 5.1A**). Pathway enrichment analysis of the 63 significantly altered serum proteins revealed "Platelet degranulation" and "Response to elevated platelet cytosolic $Ca^{2+}$" as the top two significant identified pathways in the group comparison (**Figure 5.1B)**. Generally, cases with nonanterior uveitis showed the highest levels of significantly altered proteins (q<0.05), whereas controls showed the lowest levels (**Figure 5.1C)**.

## Anterior and nonanterior uveitis

A common and clinically relevant bifurcation of pediatric uveitis phenotypes is based upon the anatomical location of disease. Because the nonanterior uveitis group has more retinal vascular involvement, we also compared the serum proteome between patients with anterior (iCAU and JIA-U, *n* = 80) versus cases with nonanterior uveitis (*n* = 74) while adjusting for sex and age. This analysis revealed 63 significantly different serum proteins, of which SERPINF1 (*q*=6.1E-4) was most significantly differentially expressed in serum, followed by Fibronectin 1 (FN1, *q*=2.6E-4), AFM (*q*=0.001), and the iron binding transport protein Transferrin (TF, *q*=0.001)(**Figure 5.2A and**

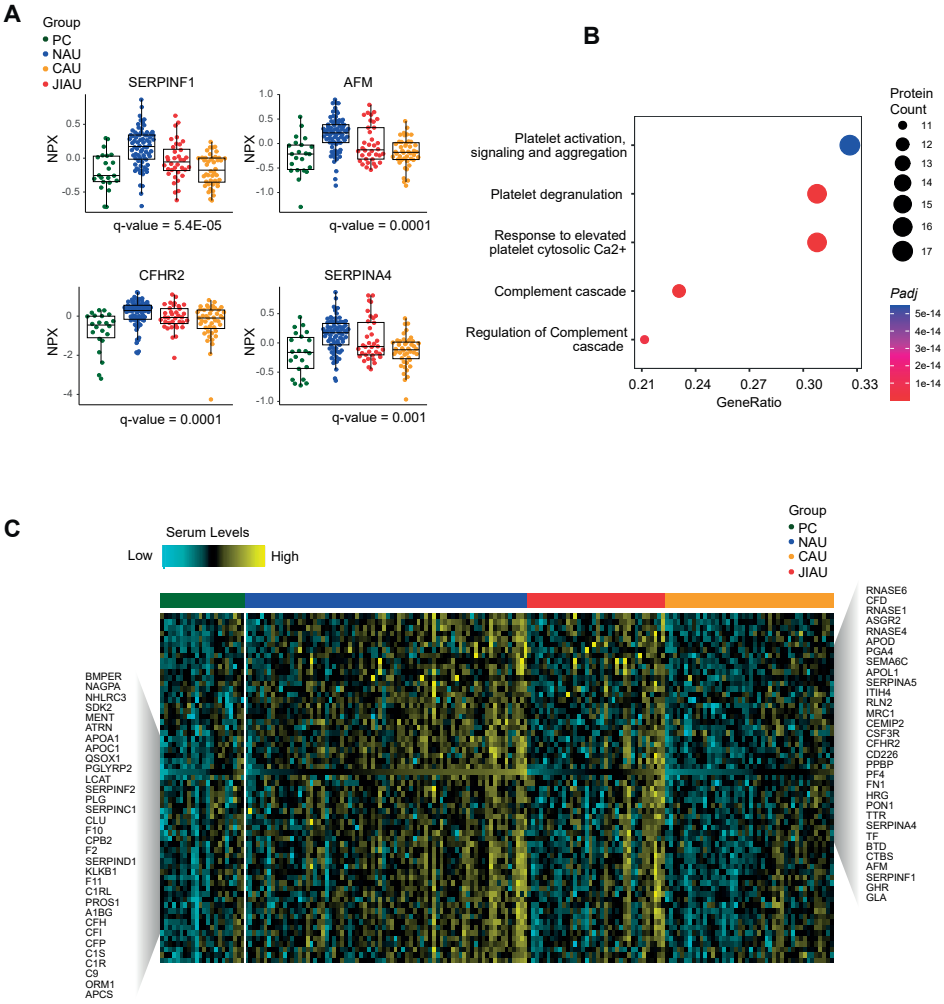


**Figure 5.1.** Serum proteome changes comparing pediatric controls (PC, *n* = 22), nonanterior uveitis patients (NAU, *n* = 74), idiopathic chronic anterior uveitis (iCAU, *n* = 36), and juvenile idiopathic arthritis associated uveitis (JIA-U, *n* = 44). **A.** Scatterplots for the top 4 differentially serum proteins ($q$<0.05). The NPX indicates the Normalized Protein eXpression data from Olink. **B.** Pathway enrichment analysis of the 63 differentially expressed proteins showing the top 5 using *Reactome* colored according to the adjusted *p*-value. **C.** Heatmap of the 63 differentially expressed proteins between the controls and uveitis subtypes. The levels for each protein analyte are shown for each of the samples in the study and are color-coded from low (cyan) to high (yellow).

**Supplemental Table S5.1**). In agreement with the analysis of the 4 disease groups, the differentially expressed proteins were generally most elevated in nonanterior uveitis **Figure 5.2C**. In total 34 proteins were identical to those detected by group comparison. These proteins were also enriched for pathways "Platelet degranulation" ($q$=6.18E-13) and "Response to elevated platelet cytosolic Ca2+" ($q$=6.18E-13). The 29 proteins uniquely identified by this analysis were enriched for "Signaling by Hippo" and "Downregulation of TGF-beta receptor signaling" as the most significant pathways, however both q-values were 0.09. A Venn diagram shows the overlapping proteins in the two analyses (**Figure 5.2C**). Pathway enrichment analysis of the 63 differently expressed serum proteins ($q$<0.05) showed a strong enrichment for the "Platelet degranulation" pathways ($q$=2.1E-9) and the "Complement cascade" pathways ($q$=2.6E-11) (**Figure 5.2B**).

## Retinal vascular involvement in nonanterior uveitis

Finally, we aimed to correlate clinical evidence for retinal vascular involvement (supported by FA and/or OCT) with serum protein markers. To this end, the nonanterior uveitis group was subdivided into active retinal vascular involvement ($n$ = 48) and non-active retinal vascular involvement ($n$ = 23). Likelihood ratio test at false discovery rate of 5% resulted in only 1 significantly different protein when adjusting for age and sex: F13B (Coagulation Factor XIII B Chain, $q$=0.006). Using a less stringent q-value of 0.1, we found 9 differentially expressed proteins. To further investigate the influence of systemic treatment on the results, we performed an additional analysis in which we corrected for the use of systemic treatment. This is shown in **Figure 5.3D**, where we plotted the protein expressions with and without correction for the use of systemic treatment. Forty-eight percent ($n$ = 23) of the patients with active retinal vascular involvement used systemic treatment, compared to 73% ($n$ = 17) of the patients with non-active retinal vascular involvement. We found the same 9 differentially expressed proteins that showed a moderate correlation (**Figure 5.3A and supplemental table S5.1)**. However, in this new analysis Apolipoprotein D (APOD) was differentially expressed instead of Serpin Family member 6 (SERPINA6). The proteins are predominantly involved in the clotting cascade as supported by pathway enrichment analysis ("Formation of Fibrin Clot" [$q$ = 1.05E-6]), such as Myomesin 3 [MYOM3] and Protein Tyrosine Phosphatase Non-Receptor 9 [PTPN9], (**Figure 5.3 and Figure 5.3B)**. Three of these proteins were unique for retinal vascular involvement (F13B, MYOM3, and PTPN9) (**Figure 5.3C**).

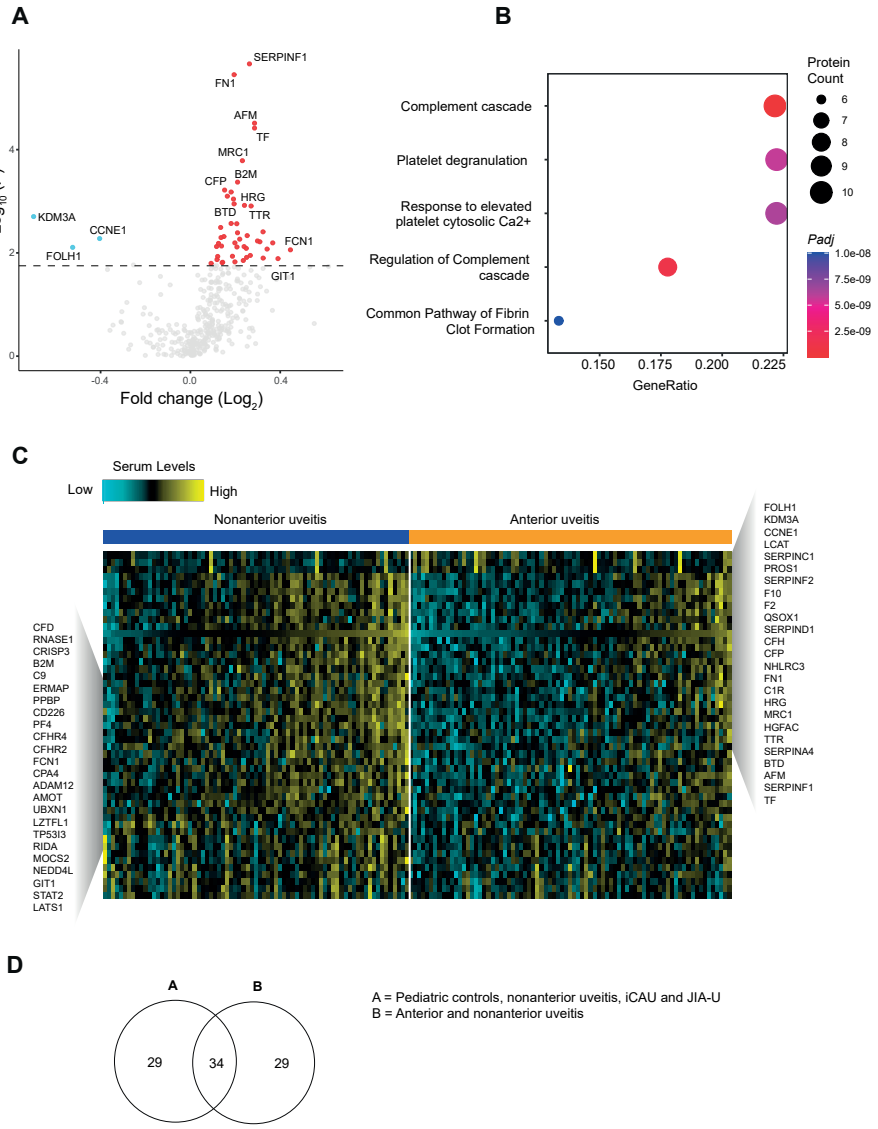


**Figure 5.2** Serum proteome changes comparing anterior (*n* = 80) versus nonanterior pediatric uveitis (*n* = 74) patients. **A.** Differential expression of serum proteins obtained from proximity extension assay (PEA) results. Differentially upregulated proteins are depicted in red, while downregulated proteins are depicted in blue. Proteins were analyzed using likelihood ratio test adjusted for age and sex and followed by a false discovery rate (FDR) multiple testing correction. The dotted line represents *q*-values of 0.05. NPX = normalized protein expression. **B.** Pathway enrichment analysis of the 63 differentially expressed proteins showing the top 5 using *Reactome* colored according to the adjusted *p*-value. **C.** Heatmap of the 63 differentially expressed proteins between the anterior and nonanterior uveitis cases. The levels for each protein analyte are shown for each of the samples in the study and are color-coded from low (cyan) to high (yellow). **D.** Venn diagram showing overlapping and unique proteins in the 4 disease group comparisons (A) and the nonanterior vs. nonanterior comparison. iCAU = idiopathic chronic anterior uveitis, JIA-U = juvenile idiopathic chronic anterior uveitis.

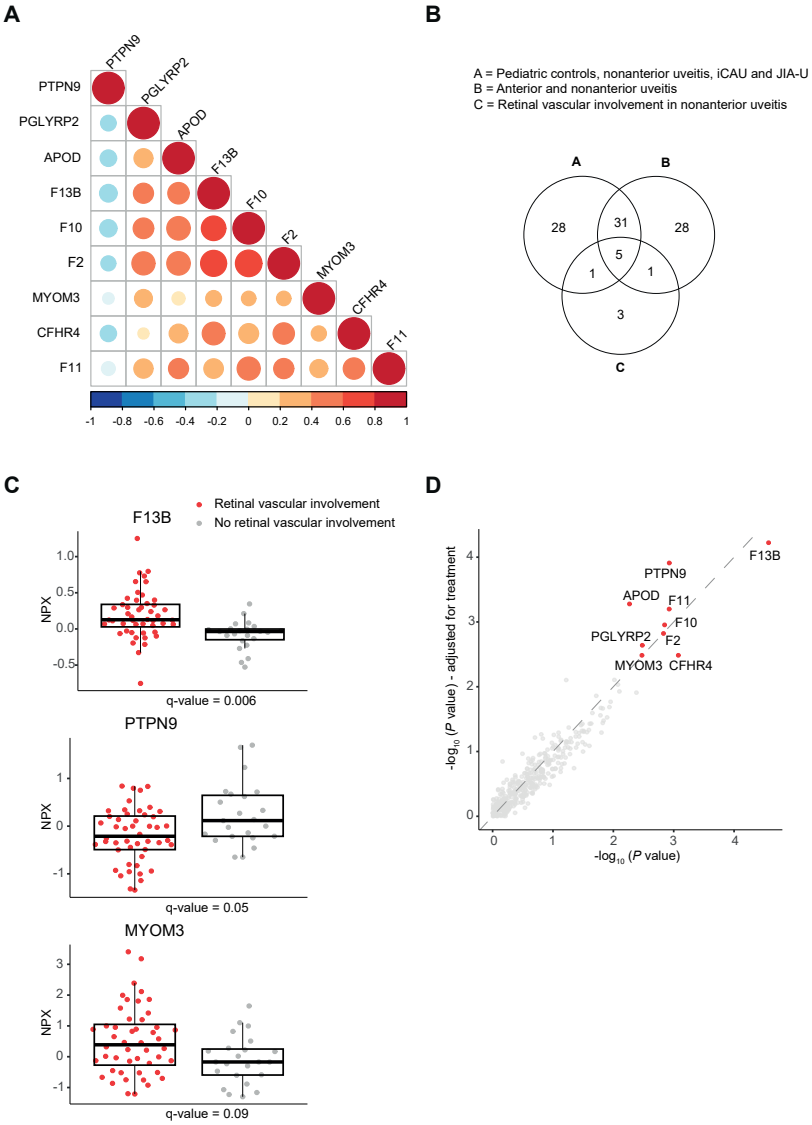


**Figure 5.3** Serum proteome changes comparing pediatric nonanterior uveitis patients with active retinal vascular involvement ($n$ = 48) and non-active retinal vascular involvement ($n$ = 23). **A.** Correlation plot showing the correlation between 9 differentially expressed serum proteins obtained from proximity extension assay (PEA) results using likelihood ratio test adjusted for age, sex and immunomodulatory treatment, and followed by a relaxed false discovery rate multiple testing correction ($q$<0.1). The NPX indicates the normalized protein expression data from Olink. **B.** The overlapping serum proteins shown in a Venn diagram of three different group comparisons: A = pediatric controls compared to nonanterior uveitis, idiopathic chronic anterior uveitis (iCAU), and juvenile idiopathic arthritis associated uveitis (JIA-U). B = anterior compared to nonanterior uveitis, C = retinal vascular involvement in nonanterior uveitis patients. **C.** Scatterplots for the 3 unique serum proteins regarding retinal vascular involvement in children with nonanterior uveitis with q<0.1, except for F13B which is q<0.05. The NPX indicates the normalized protein expression data from Olink. **D.** Protein expressions with and without correction for the use of immunomodulatory treatment.

## DISCUSSION

In this study, we used extensive PEA proteomics analysis to identify serum biomarkers to discriminate between active and non-active retinal vascular involvement in children with nonanterior uveitis. These results reveal increased serum levels of proteins related to the clotting cascade. This indicates that mediators that affect microcirculation may play a role in noninfectious pediatric uveitis. Furthermore, our study demonstrates that dysregulation of the coagulation and complement cascade contribute to the disease pathophysiology of noninfectious nonanterior uveitis in children. The question is whether the dysregulation in the coagulation and complement cascade contributes to developing retinal vascular involvement, or whether it might be a secondary effect of retinal vascular involvement in pediatric uveitis. However, the current study cannot discriminate between cause-and-effect and mainly shows associations between groups of patients.



Proteomics is a powerful screening tool for serum biomarkers in various diseases which may aid in the understanding of the pathogenesis and pathophysiology of a disease through identification of disease-associated protein profiles.[26–28] Disease biomarkers may serve agents for diagnostics, or directly serve as targets for therapeutic treatment. Previous proteomic studies have successfully identified protein signatures in serum of noninfectious uveitis that can predict the relative need for IMT.[5,7]

In our study of pediatric uveitis, we identified that disturbances of the coagulation and complement pathways are specifically related to retinal vascular involvement. Previous studies of animal models of experimental autoimmune uveitis (EAU) have also shown to be characterized by upregulation of complement and coagulation cascades.[29] Similarly, in adult patients with intermediate uveitis elevated levels of complement cascade effectors and coagulation system have been reported.[30] Platelets are well-known for their essential role in thrombus formation in response to vessel injuries.[31] However, platelets are increasingly recognized for their immune modulatory properties and role in inflammatory processes.[32,33] The storage granules of platelets contain receptors, soluble proteins and bioactive molecules that all have important pro- and anti-inflammatory functions. For example, activated platelets can trigger the activation of the complement system through these molecules. Vice versa, complement activation can induce platelet activation and aggregation, leading to enhancement of procoagulant activity.[33] Platelets may become excessively activated if this interaction between platelet and complement is not sufficiently controlled, leading to inflammatory disease. There is no evidence that the activated complement and coagulation cascade have clinical significance in pediatric uveitis patients, nor

that their effects can be reversed, but they provide an important avenue for future studies.

Idiopathic nonanterior uveitis manifests as an eye inflammatory disease often with retinal vascular involvement. In studies of uveitis, microvasculature abnormalities have been reported which are detectable through nailfold capillaroscopy, a non-invasive and highly sensitive technique to assess the systemic microcirculation.[25] We observed nailfold abnormalities, specifically more capillary dilations and a higher occurrence of ramified capillaries, in children with pediatric uveitis compared to pediatric controls (submitted for publication). Hypothetically, inflammatory markers may affect the systemic microcirculation leading to extra-ocular microvasculopathy in pediatric uveitis. Currently, there has been little molecular evidence for the presence of such vascular-active immune mediators in pediatric uveitis.

The protein that was mostly upregulated in patients with active vascular involvement was coagulation factor XIII B Chain (F13B) involved in stabilizing the fibrin clot. The F13B gene is located at a locus on chromosome 1 near the complement factor H (CFH) gene. This locus encodes regulators of the complement system. Genetic variants in chromosome 1 lead to an increased risk of developing age-related macular degeneration (AMD) driven predominantly by dysregulation of the complement system, and is also associated with central serous chorioretinopathy.[34,35] *CFH*-rs1065489 polymorphism was associated with anterior uveitis as well as uveitis recurrence.[36] There are many genetic variants or polymorphisms of the CFH-gene in the general population, and therefore it would be interesting for future research to investigate whether these polymorphisms in the CFH-gene are associated with retinal vascular involvement in idiopathic pediatric uveitis.

Another interesting topic for future research would be to investigate prospectively the predictive value of the differentially expressed proteins of retinal vascular involvement. Several patients have increased levels of the differentially expressed proteins, but do not show clinical vascular retinal involvement established by imaging (OCT and FA) at time of sampling or prior to sampling. These patients could develop retinal vascular involvement at a later stage, indicating a predictive value of the found proteins. This difference might possibly be influenced by medication use since seventy-three percent of the patient without retinal vascular involvement used immunomodulatory therapy (IMT, including corticosteroids) compared to 48% of the patients with retinal vascular involvement. Both groups had relatively higher levels of the differentially expressed proteins than patients whom did not use IMT. Also, analysis with correction for the use of IMT show that the main findings do not change.

Therefore, the immunomodulating therapy may have led to remission in those without retinal vascular involvement. In future studies, the role of medication in relation to vascular involvement and complement activation could be further investigated.

A limitation of this study is the use of serum for the proteomic analysis, and therefore several clotting factors are destroyed or consumed during the clotting process, which can complicate the interpretation. However, PEA analysis of Olink uses relative changes in proteins between samples. So even in low protein levels the relative change can be detected and analyzed.

In conclusion, we discovered an upregulation of circulating proteins with key functions in the coagulation and complement cascade in patients with retinal vascular involvement in pediatric uveitis patients. Our results indicate involvement of mediators that interact with the systemic microcirculation in nonanterior pediatric uveitis that could be interesting key targets for future studies of the immunopathogenesis of vascular involvement in pediatric noninfectious uveitis. Future identification of solid biomarkers associated with different forms of retinal vascular involvement could serve as diagnostic markers or even be potentially associated with innovative therapeutic approaches preventing vision-threatening complications and improving the general prognosis for this vulnerable group of patients.



## ACKNOWLEDGMENTS

The authors especially thank Wouter J. Venema and Aafke M. de Ligt for assisting in the data preparation.

Funding/Support: This study was generously supported by the Dutch Ophthalmology Foundation Oogfonds, the Dr. F.P. Fischer Stichting, and the ODAS Stichting.

Financial Disclosures: No financial disclosures.

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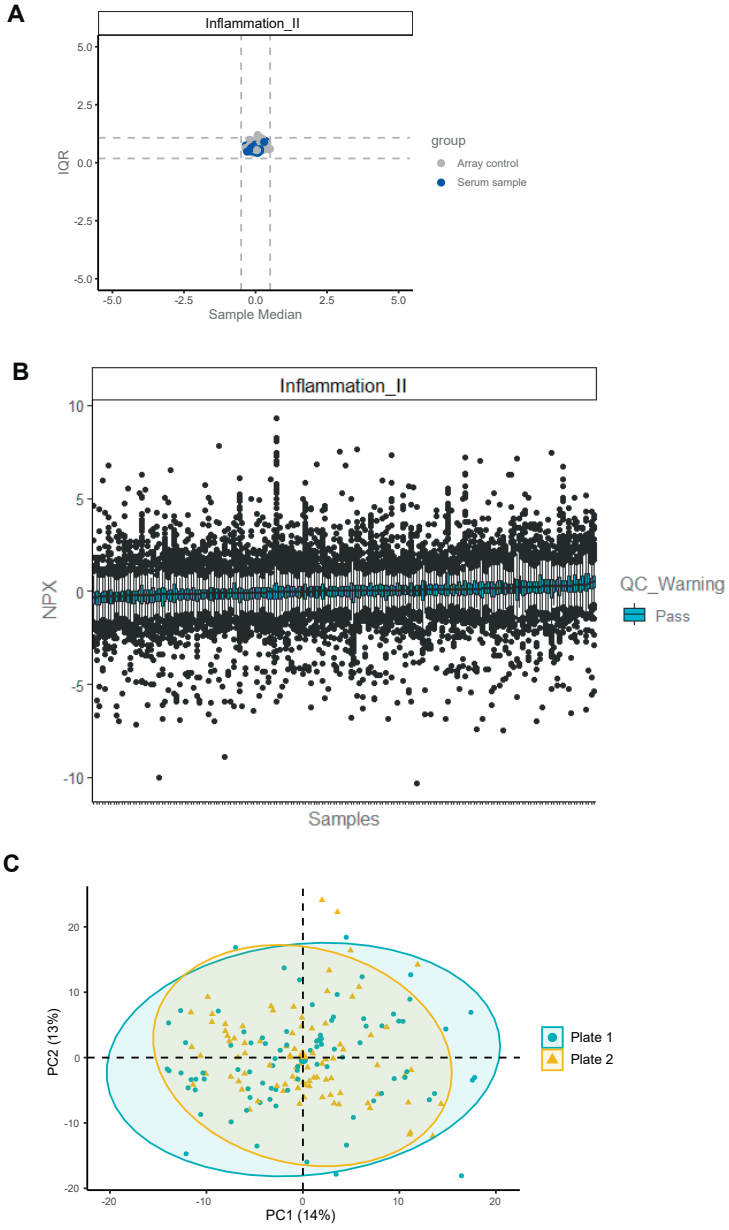
# SUPPLEMENTARY DATA




**Supplemental Figure 5.1** Quality control of the samples. **A.** Graphical overview of the distribution of the serum samples and the array controls provided by Olink. IQR = interquartile range. **B.** No proteins were detected as potential warning in the quality control provided by Olink. NPX = Normalized Protein eXpression. **C.** Principal component analysis of the two different plates to investigate potential batch effects.

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC).

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *A1BG* | Alpha-1B-glycoprotein | -0.08 (0.27) | 0.04 (0.34) | 0.08 (0.32) | 0 (0.35) | -0.12 (0.25) |
| *ABO* | Histo-blood group ABO system transferase | -2.43 (3.09) | -2.42 (3.43) | -1.21 (2.73) | -1.62 (3.06) | -1.57 (3.16) |
| *ACE* | Angiotensin-converting enzyme | 0.01 (0.4) | 0.02 (0.38) | -0.03 (0.46) | -0.09 (0.48) | -0.03 (0.47) |
| *ACHE* | Acetylcholinesterase | 0.04 (0.32) | 0.09 (0.29) | -0.04 (0.37) | -0.01 (0.43) | -0.09 (0.37) |
| *ACP1* | Low molecular weight phosphotyrosine protein phosphatase | 0.4 (1.39) | 0.45 (1.22) | -0.23 (1.35) | -0.28 (1.26) | -0.23 (1.2) |
| *ACRV1* | Acrosomal protein SP-10 | -0.05 (0.73) | 0.3 (0.8) | 0.34 (1.03) | -0.05 (0.67) | 0.09 (0.77) |
| *ACYP1* | Acylphosphatase-1 | 0.35 (1) | 0.45 (0.94) | 0.11 (1.01) | -0.01 (0.81) | -0.11 (0.75) |
| *ADAM12* | Disintegrin and metalloproteinase domain-containing protein 12 | -0.02 (0.47) | 0.24 (0.54) | 0.08 (0.66) | -0.25 (0.56) | -0.05 (0.43) |
| *ADAMTS1* | A disintegrin and metalloproteinase with thrombospondin motifs 1 | -0.15 (0.55) | 0.06 (0.44) | 0.02 (0.42) | -0.12 (0.61) | -0.05 (0.41) |
| *ADAMTS4* | A disintegrin and metalloproteinase with thrombospondin motifs 4 | 0.08 (0.53) | -0.01 (0.44) | 0.06 (0.62) | 0 (0.61) | -0.09 (0.58) |
| *ADD1* | Alpha-adducin | 0.21 (0.97) | 0.5 (0.93) | 0.11 (1.22) | -0.26 (0.99) | 0.08 (0.78) |
| *ADGRD1* | Adhesion G-protein coupled receptor D1 | 0 (0.41) | 0.25 (0.33) | 0 (0.44) | 0.04 (0.62) | -0.05 (0.4) |
| *ADH1B* | All-trans-retinol dehydrogenase | 0.39 (0.84) | 0.04 (0.54) | 0.14 (0.98) | 0.4 (0.89) | -0.15 (0.71) |
| *AFAP1* | Actin filament-associated protein 1 | 0.93 (1.62) | 0.47 (1.25) | 0.37 (1.31) | 1.06 (1.56) | 0.45 (1.53) |
| *AFM* | Afamin | -0.25 (0.4) | 0.16 (0.4) | 0.21 (0.32) | -0.01 (0.38) | -0.18 (0.29) |
| *AGT* | Angiotensinogen | -0.11 (0.16) | -0.01 (0.17) | 0.08 (0.2) | 0.12 (0.31) | -0.01 (0.25) |
| *AHSG* | Alpha-2-HS-glycoprotein | -0.06 (0.29) | 0.04 (0.25) | 0.04 (0.28) | -0.07 (0.3) | -0.06 (0.29) |

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC). (*continued*)

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *AKAP12* | A-kinase anchor protein 12 | 0.07 (0.42) | 0.1 (0.43) | -0.14 (0.42) | -0.03 (0.53) | -0.04 (0.41) |
| *AKR7L* | Aflatoxin B1 aldehyde reductase member 4 | 0.49 (0.94) | -0.03 (0.52) | 0.05 (1.05) | 0.34 (1.02) | 0 (0.99) |
| *ALPI* | Intestinal-type alkaline phosphatase | -0.53 (1.5) | 0.31 (1.39) | 0.12 (1.32) | -0.02 (1.92) | 0.02 (1.65) |
| *AMOT* | Angiomotin | -0.03 (0.53) | 0.25 (0.4) | -0.02 (0.61) | -0.25 (0.71) | -0.14 (0.53) |
| *AMY1A_ AMY1B_ AMY1C* | Alpha-amylase 1A_Alpha-amylase 1B_Alpha-amylase 1C | -0.04 (0.12) | 0.01 (0.13) | -0.01 (0.13) | -0.01 (0.11) | 0 (0.13) |
| *ANKMY2* | Ankyrin repeat and MYND domain-containing protein 2 | 0.01 (0.14) | 0.01 (0.14) | -0.01 (0.12) | 0.03 (0.13) | -0.02 (0.13) |
| *ANXA1* | Annexin A1 | 0 (0.07) | 0.02 (0.07) | -0.02 (0.07) | -0.02 (0.08) | 0.01 (0.07) |
| *APCS* | Serum amyloid P-component | -0.01 (0.06) | -0.01 (0.05) | 0 (0.05) | -0.01 (0.06) | 0 (0.05) |
| *APOA1* | Apolipoprotein A-I | 0.01 (0.63) | -0.13 (0.42) | 0.12 (0.6) | -0.21 (0.55) | -0.09 (0.53) |
| *APOA2* | Apolipoprotein A-II | 0.25 (0.76) | 0.4 (1.21) | 0.01 (1.1) | -0.21 (1.02) | -0.2 (0.98) |
| *APOA4* | Apolipoprotein A-IV | 0.14 (0.55) | 0.15 (0.56) | 0.04 (0.52) | 0.03 (0.54) | 0.07 (0.53) |
| *APOB* | Apolipoprotein B-100 | -0.42 (0.55) | -0.07 (0.53) | 0.19 (0.47) | -0.08 (0.52) | -0.15 (0.43) |
| *APOC1* | Apolipoprotein C-I | -0.09 (0.31) | 0.09 (0.29) | 0.03 (0.27) | 0.05 (0.33) | -0.08 (0.24) |
| *APOD* | Apolipoprotein D | 0.1 (0.73) | 0.15 (0.55) | 0.01 (0.73) | 0.24 (1.26) | -0.19 (0.56) |
| *APOE* | Apolipoprotein E | -0.05 (0.41) | 0.08 (0.4) | -0.03 (0.56) | 0.08 (0.55) | -0.01 (0.38) |
| *APOF* | Apolipoprotein F | -0.11 (0.43) | -0.1 (0.54) | 0.09 (0.38) | 0.15 (0.53) | -0.15 (0.49) |
| *APOL1* | Apolipoprotein L1 | -0.21 (0.62) | 0.06 (0.36) | 0.02 (0.46) | -0.02 (0.48) | -0.15 (0.38) |
| *APPL2* | DCC-interacting protein 13-beta | -0.19 (0.35) | 0.09 (0.54) | 0.19 (0.46) | 0.18 (0.53) | -0.04 (0.29) |
| *ARHGAP45* | Rho GTPase-activating protein 45 | -0.08 (0.33) | 0.06 (0.43) | 0.05 (0.41) | 0.12 (0.37) | -0.03 (0.24) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *ASGR2* | Asialoglycoprotein receptor 2 | -0.17 (0.44) | -0.04 (0.18) | 0 (0.36) | -0.08 (0.37) | -0.01 (0.21) |
| *ATRN* | Attractin, Isoform 2 | -0.22 (0.5) | -0.05 (0.45) | 0.27 (0.75) | 0.25 (0.96) | -0.13 (0.47) |
| *B2M* | Beta-2-microglobulin | 0.47 (0.9) | 0.3 (1.34) | -0.01 (1.08) | -0.05 (0.96) | -0.37 (1.24) |
| *BABAM1* | BRISC and BRCA1-A complex member 1 | 0.26 (0.61) | 0.32 (0.78) | 0.02 (0.65) | -0.14 (0.71) | -0.09 (0.67) |
| *BAG4* | BAG family molecular chaperone regulator 4 | -0.18 (0.46) | 0.02 (0.43) | 0.19 (0.42) | 0.13 (0.49) | -0.11 (0.44) |
| *BCHE* | Cholinesterase | -0.03 (0.35) | 0.02 (0.26) | 0.05 (0.28) | 0.01 (0.41) | -0.15 (0.27) |
| *BCL2L15* | Bcl-2-like protein 15 | 0.14 (0.49) | 0.08 (0.3) | 0.22 (0.38) | 0.02 (0.32) | -0.1 (0.3) |
| *BLNK* | B-cell linker protein | 0.09 (0.58) | -0.05 (0.88) | 0 (0.76) | 0.03 (0.8) | 0.02 (0.67) |
| *BMP10* | Bone morphogenetic protein 10 | 0.15 (0.67) | 0.15 (0.54) | 0.04 (0.73) | -0.25 (0.84) | 0.02 (0.57) |
| *BMPER* | BMP-binding endothelial regulator protein | -0.09 (0.38) | 0.13 (0.32) | 0.03 (0.27) | -0.01 (0.38) | -0.04 (0.27) |
| *BNIP3L* | BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like | -0.07 (1.19) | 0.16 (1.02) | 0.18 (1.16) | 0.09 (1.3) | 0.09 (1.2) |
| *BTD* | Biotinidase | 0.23 (0.68) | -0.06 (0.52) | 0.1 (0.77) | -0.08 (0.75) | 0.47 (0.84) |
| *C1QL2* | Complement C1q-like protein 2 | 0.07 (0.33) | 0.07 (0.38) | -0.1 (0.47) | 0 (0.44) | -0.06 (0.36) |
| *C1QTNF5* | Complement C1q tumor necrosis factor-related protein 5 | -0.05 (0.35) | 0.06 (0.23) | 0.13 (0.36) | 0.06 (0.42) | -0.06 (0.28) |
| *C1QTNF9* | Complement C1q and tumor necrosis factor-related protein 9A | 0.26 (0.74) | 0.3 (0.8) | 0.08 (0.67) | 0.14 (0.79) | -0.08 (0.76) |
| *C1R* | Complement C1r subcomponent | -0.07 (0.28) | 0.1 (0.38) | 0.14 (0.34) | 0.03 (0.48) | -0.15 (0.25) |
| *C1RL* | Complement C1r subcomponent-like protein | 0.05 (0.57) | -0.02 (0.53) | 0.01 (0.55) | -0.22 (0.61) | 0.14 (0.6) |
| *C1S* | Complement C1s subcomponent | -0.03 (0.36) | 0.05 (0.41) | 0.1 (0.38) | -0.03 (0.5) | -0.07 (0.31) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *C3* | Complement C3 | -0.13 (0.54) | 0.16 (0.47) | -0.02 (0.5) | 0.02 (0.66) | -0.18 (0.64) |
| *C5* | Complement C5 | -0.06 (0.22) | 0.07 (0.22) | 0.04 (0.25) | -0.07 (0.32) | -0.08 (0.26) |
| *C7* | Complement component C7 | -0.15 (0.34) | 0.08 (0.25) | 0.09 (0.33) | 0.03 (0.28) | -0.04 (0.34) |
| *C8B* | Complement component C8 beta chain | -0.09 (0.27) | 0.09 (0.3) | 0.1 (0.35) | 0.07 (0.32) | -0.09 (0.31) |
| *C9* | Complement component C9 | -0.03 (0.24) | -0.05 (0.28) | 0.2 (0.52) | 0.22 (0.7) | -0.04 (0.3) |
| *CA8* | Carbonic anhydrase-related protein | -0.13 (0.28) | -0.04 (0.29) | 0.02 (0.27) | 0.02 (0.26) | -0.04 (0.17) |
| *CACYBP* | Calcyclin-binding protein | -0.15 (0.29) | -0.07 (0.27) | 0.05 (0.5) | 0.05 (0.39) | -0.03 (0.25) |
| *CASP9* | Caspase-9 | -0.09 (0.32) | 0.01 (0.32) | 0.07 (0.4) | -0.05 (0.49) | 0.02 (0.38) |
| *CAT* | Catalase | -0.21 (0.52) | -0.13 (0.46) | 0.2 (0.42) | -0.15 (0.47) | -0.07 (0.44) |
| *CCNE1* | G1/S-specific cyclin-E1 | -0.09 (0.32) | 0.07 (0.43) | 0.1 (0.37) | 0.01 (0.35) | 0.11 (0.61) |
| *CD226* | CD226 antigen | 0.48 (0.93) | 0.44 (0.94) | 0.03 (0.87) | 0.16 (1.05) | -0.15 (1.07) |
| *CD300A* | CMRF35-like molecule 8 | 0.4 (1.19) | 0.44 (1.29) | -0.18 (1.08) | -0.11 (1.15) | -0.12 (1.21) |
| *CD36* | Platelet glycoprotein 4 | 0.32 (0.93) | 0.42 (0.86) | 0.19 (0.99) | -0.12 (0.74) | 0.11 (0.76) |
| *CD3G* | T-cell surface glycoprotein CD3 gamma chain | -0.12 (0.36) | 0.14 (1.02) | 0.19 (0.93) | 0.79 (1.78) | 0.39 (0.93) |
| *CD5L* | CD5 antigen-like | -0.05 (0.53) | 0.12 (0.43) | 0.13 (0.4) | -0.02 (0.37) | -0.18 (0.49) |
| *CD7* | T-cell antigen CD7 | 0.01 (0.39) | 0.02 (0.35) | 0.08 (0.41) | -0.03 (0.39) | -0.1 (0.35) |
| *CD72* | B-cell differentiation antigen CD72 | -0.01 (0.5) | -0.01 (0.4) | 0.04 (0.47) | -0.03 (0.42) | 0.01 (0.48) |
| *CEBPA* | CCAAT/enhancer-binding protein alpha | 0.54 (1.44) | 0.25 (1.61) | 0.34 (1.22) | 0.36 (1.47) | 0.17 (1.52) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *CELSR2* | Cadherin EGF LAG seven-pass G-type receptor 2 | 0.03 (0.41) | 0.02 (0.53) | 0.1 (0.61) | -0.01 (0.61) | -0.12 (0.46) |
| *CEMIP2* | Cell surface hyaluronidase | 0.16 (0.49) | -0.2 (0.45) | -0.08 (0.45) | 0.04 (0.37) | -0.1 (0.37) |
| *CFB* | Complement factor B | 0.31 (0.6) | -0.15 (0.55) | -0.02 (0.65) | -0.17 (0.56) | -0.01 (0.5) |
| *CFD* | Complement factor D | -0.1 (0.51) | 0.2 (0.52) | 0.19 (0.6) | 0.17 (0.75) | 0.09 (0.73) |
| *CFH* | Complement factor H | 0.17 (0.32) | 0.08 (0.3) | -0.25 (0.78) | -0.4 (1.17) | -0.04 (0.27) |
| *CFHR2* | Complement factor H-related protein 2 | -0.08 (0.23) | 0.05 (0.38) | 0.1 (0.31) | 0.02 (0.39) | -0.09 (0.25) |
| *CFHR4* | Complement factor H-related protein 4 | -0.06 (0.54) | -0.18 (0.55) | 0.17 (0.45) | 0.04 (0.51) | -0.06 (0.42) |
| *CFHR5* | Complement factor H-related protein 5 | -0.08 (0.28) | 0.14 (0.36) | 0.15 (0.32) | 0.03 (0.35) | -0.1 (0.23) |
| *CFI* | Complement factor I | -0.14 (0.32) | 0.06 (0.25) | 0.15 (0.31) | -0.03 (0.35) | -0.09 (0.26) |
| *CFP* | Properdin | -0.77 (1.02) | -0.2 (0.89) | 0.26 (0.53) | -0.07 (0.63) | -0.3 (0.87) |
| *CGB3_ CGB5_ CGB8* | Choriogonadotropin subunit beta 3 | -0.11 (0.58) | 0.12 (0.41) | 0.24 (0.62) | -0.18 (0.57) | -0.06 (0.67) |
| *CHAD* | Chondroadherin | -0.24 (0.41) | -0.08 (0.47) | 0.14 (0.49) | -0.01 (0.56) | -0.06 (0.43) |
| *CLEC12A* | C-type lectin domain family 12 member A | -0.19 (0.36) | -0.07 (0.38) | 0.12 (0.31) | -0.02 (0.41) | -0.06 (0.3) |
| *CLEC3B* | Tetranectin | -0.12 (0.25) | 0.06 (0.26) | 0.12 (0.25) | 0 (0.31) | -0.1 (0.23) |
| *CLU* | Clusterin | -0.12 (1.29) | -0.13 (0.81) | -0.15 (1.05) | -0.11 (1.03) | -0.17 (0.75) |
| *COL5A1* | Collagen alpha-1(V) chain | -0.12 (0.47) | 0.02 (0.4) | -0.19 (0.55) | -0.18 (0.6) | -0.1 (0.44) |
| *CPA4* | Carboxypeptidase A4 | 0.07 (0.49) | -0.07 (0.44) | 0.15 (0.5) | 0.11 (0.44) | 0.02 (0.53) |
| *CPB2* | Carboxypeptidase B2 | -0.03 (0.27) | 0.11 (0.24) | 0.01 (0.24) | -0.01 (0.36) | -0.07 (0.25) |
| *CPOX* | Oxygen-dependent coproporphyrinogen-III oxidase, mitochondrial | -0.07 (0.26) | 0.01 (0.33) | 0.11 (0.35) | 0.08 (0.37) | -0.11 (0.21) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *CR1* | Complement receptor type 1 | -0.02 (0.68) | 0.09 (0.64) | -0.18 (0.72) | -0.2 (0.74) | -0.04 (0.58) |
| *CRELD1* | Protein disulfide isomerase CRELD1 | 0 (0.55) | 0.08 (0.44) | 0.22 (0.46) | -0.24 (0.9) | -0.08 (0.43) |
| *CRISP3* | Cysteine-rich secretory protein 3 | -0.22 (0.36) | 0 (0.38) | 0.12 (0.3) | 0.01 (0.34) | -0.06 (0.27) |
| *CSF1R* | Macrophage colony-stimulating factor 1 receptor | -0.04 (0.44) | -0.07 (0.31) | 0.01 (0.47) | 0.04 (0.55) | 0.05 (0.39) |
| *CSF2RB* | Cytokine receptor common subunit beta | 0.11 (0.47) | -0.16 (0.47) | 0.04 (0.51) | 0.02 (0.58) | 0.06 (0.41) |
| *CSF3R* | Granulocyte colony-stimulating factor receptor | -0.09 (0.51) | 0.12 (0.48) | 0.1 (0.37) | -0.1 (0.52) | -0.1 (0.29) |
| *CSH1* | Chorionic somatomammotropin hormone 1 | -0.05 (0.24) | 0.14 (0.24) | 0.07 (0.31) | -0.1 (0.35) | -0.09 (0.28) |
| *CSNK1D* | Casein kinase I isoform delta | 0.09 (0.48) | -0.13 (0.42) | 0.05 (0.62) | 0.07 (0.49) | -0.07 (0.44) |
| *CST1* | Cystatin-SN | 0.04 (0.79) | -0.02 (0.65) | -0.05 (0.76) | -0.04 (0.57) | -0.16 (0.6) |
| *CTBS* | Di-N-acetylchitobiase | -0.24 (0.44) | 0.11 (0.3) | 0.08 (0.44) | -0.01 (0.59) | -0.05 (0.44) |
| *CTSE* | Cathepsin E | -0.26 (0.87) | -0.1 (1.34) | -0.33 (1.29) | 0.04 (1.18) | -0.24 (0.95) |
| *DAAM1* | Disheveled-associated activator of morphogenesis 1 | 0.27 (0.82) | -0.16 (1.42) | -0.08 (1.13) | 0.21 (1.4) | 0.12 (1.01) |
| *DAND5* | DAN domain family member 5 | -0.2 (1.19) | -0.4 (1.35) | -0.05 (1.28) | -0.01 (1.03) | -0.08 (1.14) |
| *DAPK2* | Death-associated protein kinase 2 | -0.17 (0.38) | 0.16 (0.45) | 0.09 (0.33) | 0.08 (0.46) | -0.16 (0.26) |
| *DBH* | Dopamine beta-hydroxylase | -0.3 (0.54) | 0.07 (0.58) | 0.16 (0.6) | 0.05 (0.69) | -0.04 (0.54) |
| *DBN1* | Drebrin | 0.24 (0.8) | 0.05 (1) | -0.19 (0.97) | -0.14 (0.9) | -0.55 (1.2) |
| *DCTD* | Deoxycytidylate deaminase | -0.17 (1.84) | -0.4 (1.77) | -1.03 (2.07) | -0.17 (1.8) | -0.84 (2.07) |
| *DDI2* | Protein DDI1 homolog 2 | 0.08 (0.81) | 0.16 (0.72) | 0.04 (0.91) | -0.04 (1.01) | 0.13 (1.02) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *DDX39A* | ATP-dependent RNA helicase DDX39A | -0.45 (0.98) | -0.08 (0.66) | -0.09 (1.16) | -0.45 (1.48) | -0.22 (1.36) |
| *DDX4* | Probable ATP-dependent RNA helicase DDX4 | 0.07 (0.6) | 0.38 (0.74) | 0.11 (0.63) | 0.16 (0.85) | -0.06 (0.6) |
| *DEFB103A_ DEFB103B* | Beta-defensin 103 | 0.47 (0.8) | 0.39 (1.08) | 0.11 (0.92) | -0.16 (1) | -0.04 (1.05) |
| *DENR* | Density-regulated protein | 0.28 (1.33) | 0.58 (1.3) | 0.07 (1.44) | -0.45 (1.44) | 0.08 (1.02) |
| *DGKA* | Diacylglycerol kinase alpha | 0.14 (0.42) | 0.06 (0.29) | -0.02 (0.4) | -0.02 (0.38) | 0.05 (0.41) |
| *DIPK2B* | Divergent protein kinase domain 2B | 0.03 (1.86) | -0.2 (1.95) | 0.09 (1.31) | -0.06 (1.53) | 0.19 (1.85) |
| *DNAJB2* | DnaJ homolog subfamily B member 2 | -0.22 (0.77) | -0.04 (0.63) | 0.2 (0.86) | 0.18 (0.87) | 0.08 (0.87) |
| *DNAJB6* | DnaJ homolog subfamily B member 6 | 0.4 (0.86) | 0.39 (0.92) | 0.01 (0.96) | -0.19 (0.72) | -0.05 (0.61) |
| *DTD1* | D-aminoacyl-tRNA deacylase 1 | 0.15 (0.68) | 0.29 (0.55) | 0.11 (0.54) | 0 (0.63) | -0.02 (0.9) |
| *ECM1* | Extracellular matrix protein 1 | 0 (0.41) | 0.05 (0.27) | -0.02 (0.35) | -0.09 (0.43) | 0 (0.36) |
| *EDNRB* | Endothelin receptor type B | 0.33 (0.92) | 0.41 (0.83) | 0.05 (0.99) | -0.04 (0.82) | -0.11 (0.8) |
| *EIF4E* | Eukaryotic translation initiation factor 4E | 0.26 (0.89) | 0.26 (0.96) | -0.08 (0.82) | 0.15 (1.03) | -0.29 (1.08) |
| *EPHA4* | Ephrin type-A receptor 4 | 0.42 (0.78) | 0.52 (1.32) | 0.17 (1.08) | -0.04 (0.99) | -0.03 (1.11) |
| *ERMAP* | Erythroid membrane-associated protein | -0.22 (0.52) | -0.1 (0.42) | 0 (0.63) | -0.2 (0.64) | 0.05 (0.56) |
| *ERP29* | Endoplasmic reticulum resident protein 29 | 0.24 (0.35) | 0 (0.31) | -0.08 (0.44) | 0.04 (0.67) | -0.06 (0.26) |
| *ESR1* | Estrogen receptor | 0.5 (1.51) | 0.61 (1.55) | 0.14 (1.69) | -0.32 (1.44) | -0.07 (1.15) |
| *EVI5* | Ecotropic viral integration site 5 protein homolog | 0.2 (0.53) | -0.07 (0.25) | -0.1 (0.4) | 0.02 (0.43) | -0.06 (0.38) |
| *F10* | Coagulation factor X | 0.06 (0.51) | 0.21 (0.46) | 0.13 (0.42) | 0.02 (0.43) | -0.2 (0.63) |
| *F11* | Coagulation factor XI | 0.44 (0.82) | 0.27 (0.81) | -0.04 (0.74) | 0.01 (0.91) | -0.24 (0.99) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *F12* | Coagulation factor XII | -0.71 (2.01) | 0.01 (1.52) | 0.11 (1.45) | 0.04 (2.42) | 0.36 (1.8) |
| *F13B* | Coagulation factor XIII B chain | 0.41 (0.64) | 0.07 (1.11) | -0.01 (0.85) | 0.07 (0.97) | -0.1 (1.03) |
| *F2* | Prothrombin | -0.03 (0.27) | 0.03 (0.3) | 0.11 (0.28) | 0.01 (0.38) | -0.11 (0.25) |
| *FCN1* | Ficolin-1 | -0.28 (0.65) | 0.19 (0.57) | 0.12 (0.68) | 0.13 (0.59) | -0.14 (0.54) |
| *FGA* | Fibrinogen alpha chain | -0.1 (0.64) | 0.09 (0.5) | -0.06 (0.6) | -0.14 (0.71) | -0.15 (0.39) |
| *FGF12* | Fibroblast growth factor 12 | -0.01 (0.24) | 0.14 (0.44) | 0.08 (0.26) | 0.03 (0.34) | -0.07 (0.31) |
| *FGF16* | Fibroblast growth factor 16 | -0.07 (0.24) | 0.03 (0.27) | 0.1 (0.27) | 0.04 (0.3) | -0.13 (0.22) |
| *FGF20* | Fibroblast growth factor 20 | -0.11 (0.62) | 0.16 (0.78) | 0.17 (0.86) | -0.03 (0.89) | -0.45 (1.11) |
| *FGF6* | Fibroblast growth factor 6 | 0.13 (0.55) | 0 (0.1) | 0.02 (0.19) | 0.03 (0.11) | 0.04 (0.16) |
| *FGFR4* | Fibroblast growth factor receptor 4 | 0.63 (0.87) | 0.2 (0.98) | -0.01 (1.13) | 0.23 (0.8) | 0.19 (0.62) |
| *FGL1* | Fibrinogen-like protein 1 | -0.21 (0.68) | -0.15 (0.65) | 0.13 (1.23) | -0.02 (0.65) | -0.01 (0.51) |
| *FN1* | Fibronectin | 0.05 (0.32) | 0.09 (0.53) | 0.12 (0.49) | 0.04 (0.28) | 0.09 (0.36) |
| *FOLH1* | Glutamate carboxypeptidase 2 | 0.1 (0.87) | 0.01 (0.47) | 0.1 (0.62) | 0.07 (0.7) | 0.04 (0.49) |
| *FOXJ3* | Forkhead box protein J3 | 0.21 (0.71) | 0.17 (0.66) | 0.08 (0.57) | -0.27 (0.6) | -0.01 (0.56) |
| *GAD2* | Glutamate decarboxylase 2 | -0.15 (1.08) | -0.04 (0.77) | 0.08 (0.78) | -0.11 (0.99) | 0.09 (0.81) |
| *GAPDH* | Glyceraldehyde-3-phosphate dehydrogenase | -0.06 (0.18) | 0.18 (0.27) | 0.14 (0.25) | -0.1 (0.26) | -0.01 (0.21) |
| *GC* | Vitamin D-binding protein | -0.32 (0.69) | 0.29 (1.51) | 0.37 (1.45) | 1.13 (2.48) | 0.61 (1.69) |
| *GCHFR* | GTP cyclohydrolase 1 feedback regulatory protein | 0.19 (0.62) | 0.15 (0.53) | -0.09 (0.69) | -0.24 (0.79) | -0.03 (0.63) |
| *GHR* | Growth hormone receptor | 0.07 (0.7) | 0.06 (0.66) | -0.1 (0.77) | 0.27 (0.71) | 0.17 (0.86) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *GIMAP7* | GTPase IMAP family member 7 | 0.06 (0.36) | 0.09 (0.38) | -0.42 (1.11) | -0.85 (1.58) | -0.08 (0.36) |
| *GIPR* | Gastric inhibitory polypeptide receptor | -0.03 (0.33) | 0.01 (0.38) | 0.03 (0.39) | 0.02 (0.36) | -0.06 (0.38) |
| *GIT1* | ARF GTPase-activating protein GIT1 | 0.28 (0.57) | 0.15 (0.44) | 0.16 (0.71) | -0.01 (0.49) | -0.08 (0.6) |
| *GLA* | Alpha-galactosidase A | -0.28 (0.61) | 0.12 (0.65) | 0.2 (0.43) | 0.05 (0.45) | -0.2 (0.42) |
| *GLI2* | Zinc finger protein GLI2 | 0.14 (0.41) | -0.03 (0.33) | -0.04 (0.4) | -0.06 (0.42) | 0.03 (0.45) |
| *GLRX5* | Glutaredoxin-related protein 5, mitochondrial | -1.13 (2.38) | 0.31 (1.29) | -0.5 (2.07) | -0.47 (2.04) | -1.18 (2.5) |
| *GMPR2* | GMP reductase 2 | 0.44 (0.92) | 0.54 (1.23) | 0.28 (1.18) | -0.26 (1.15) | 0.05 (0.89) |
| *GNPDA2* | Glucosamine-6-phosphate isomerase 2 | -0.15 (0.38) | 0.01 (0.3) | 0.06 (0.29) | 0.05 (0.41) | -0.21 (0.39) |
| *GP5* | Platelet glycoprotein V | -0.09 (0.26) | 0.12 (0.52) | 0.04 (0.43) | 0.3 (1.04) | 0.11 (0.49) |
| *GPI* | Glucose-6-phosphate isomerase | 0.39 (0.63) | 0.13 (0.93) | 0 (0.85) | -0.14 (0.82) | -0.32 (0.68) |
| *GSN* | Gelsolin | 0.36 (0.86) | 0.42 (0.94) | 0.02 (1.07) | -0.33 (0.96) | 0.01 (0.65) |
| *GSR* | Glutathione reductase, mitochondrial | 0.1 (0.38) | 0.28 (0.4) | -0.1 (0.41) | -0.14 (0.5) | 0.02 (0.42) |
| *HDAC8* | Histone deacetylase 8 | 0.15 (0.24) | 0.11 (0.27) | 0.01 (0.22) | -0.01 (0.24) | 0 (0.2) |
| *HEG1* | Protein HEG homolog 1 | 0.1 (0.61) | 0.08 (0.54) | 0.01 (0.64) | -0.12 (0.6) | -0.02 (0.53) |
| *HGFAC* | Hepatocyte growth factor activator | -0.06 (0.21) | 0.03 (0.21) | 0.1 (0.25) | 0.06 (0.37) | -0.06 (0.21) |
| *HIF1A* | Hypoxia-inducible factor 1-alpha | 0.07 (0.28) | 0.08 (0.32) | -0.01 (0.33) | -0.1 (0.37) | 0 (0.32) |
| *HMCN2* | Hemicentin-2 | -0.27 (2.01) | 0.1 (2.79) | -0.1 (2.32) | 0.22 (2.43) | 0.25 (1.37) |
| *HRG* | Histidine-rich glycoprotein | 0.05 (0.38) | -0.02 (0.21) | 0.04 (0.35) | 0 (0.42) | -0.08 (0.31) |
| *HS6ST2* | Heparan-sulfate 6-O-sulfotransferase 2 | 0.04 (0.37) | 0.06 (0.37) | 0.02 (0.34) | -0.07 (0.51) | -0.15 (0.33) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *IDO1* | Indoleamine 2,3-dioxygenase 1 | 0.65 (1.77) | 0 (0.45) | 0.31 (0.97) | 0.28 (1.03) | 0.37 (0.89) |
| *IGFL4* | Insulin growth factor-like family member 4 | 0.18 (0.62) | 0.11 (0.7) | 0 (0.74) | 0.11 (1.31) | -0.03 (0.56) |
| *IGLC2* | Immunoglobulin lambda constant 2 | -0.11 (0.49) | 0.12 (0.46) | 0.13 (0.34) | -0.02 (0.35) | -0.18 (0.41) |
| *IL12RB2* | Interleukin-12 receptor subunit beta-2 | 0.29 (0.6) | 0.06 (0.37) | 0 (0.5) | -0.06 (0.59) | -0.03 (0.37) |
| *IL20RB* | Interleukin-20 receptor subunit beta | 0.7 (1.18) | -0.14 (1.06) | 0.08 (1.15) | -0.12 (0.85) | 0.04 (0.94) |
| *IL21R* | Interleukin-21 receptor | 0.47 (1.77) | -0.39 (0.95) | 0.16 (1.07) | 0.66 (1.89) | 0.13 (1.2) |
| *IL31* | Interleukin-31 | 0.06 (0.26) | 0.03 (0.25) | 0.07 (0.29) | -0.01 (0.18) | -0.03 (0.23) |
| *IL31RA* | Interleukin-31 receptor subunit alpha | 0.19 (0.77) | -0.09 (0.58) | 0.05 (0.71) | 0.34 (0.91) | 0.07 (0.81) |
| *IL36A* | Interleukin-36 alpha | 0.06 (0.81) | 0.07 (0.45) | -0.01 (0.55) | 0.09 (0.6) | 0.08 (0.63) |
| *IL36G* | Interleukin-36 gamma | 0.5 (1.05) | -0.11 (0.45) | 0.09 (0.9) | 0.1 (0.6) | 0.25 (0.95) |
| *INHBB* | Inhibin beta B chain | 0.38 (1.32) | 0.05 (0.86) | 0.16 (0.93) | 0.08 (0.85) | 0.26 (0.81) |
| *INSR* | Insulin receptor | 0.36 (0.75) | 0.08 (0.75) | -0.24 (0.86) | -0.06 (0.84) | 0.04 (0.84) |
| *ITGA2* | Integrin alpha-2 | -0.48 (3.14) | -0.54 (1.95) | -0.04 (1.33) | -0.39 (1.88) | -0.19 (1.47) |
| *ITGAL* | Integrin alpha-L | 0.11 (0.8) | -0.05 (0.51) | -0.02 (0.75) | 0.03 (0.6) | 0.29 (0.8) |
| *ITIH1* | Inter-alpha-trypsin inhibitor heavy chain H1 | -0.03 (0.09) | 0.01 (0.1) | 0.01 (0.11) | 0.01 (0.09) | -0.01 (0.1) |
| *ITIH4* | Inter-alpha-trypsin inhibitor heavy chain H4 | 0 (0.1) | 0 (0.1) | -0.01 (0.08) | 0 (0.1) | -0.03 (0.08) |
| *JAM3* | Junctional adhesion molecule C | -0.01 (0.07) | -0.01 (0.06) | 0.01 (0.06) | 0.01 (0.07) | -0.01 (0.07) |
| *KDM3A* | Lysine-specific demethylase 3A | -0.02 (0.09) | -0.01 (0.07) | 0.01 (0.07) | -0.01 (0.07) | 0 (0.07) |
| *KLK7* | Kallikrein-7 | -0.06 (0.58) | 0.07 (0.63) | 0.22 (0.63) | 0.12 (0.67) | -0.08 (0.58) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *KLKB1* | Plasma kallikrein | 0 (0.1) | -0.02 (0.13) | 0.02 (0.15) | 0 (0.13) | -0.02 (0.15) |
| *KLRF1* | Killer cell lectin-like receptor subfamily F member 1 | 0.09 (0.59) | 0.16 (0.53) | -0.16 (0.49) | -0.13 (0.72) | 0 (0.48) |
| *LATS1* | Serine/threonine-protein kinase LATS1 | 0.25 (0.57) | -0.06 (0.54) | 0.04 (0.76) | 0.16 (0.9) | -0.15 (0.55) |
| *LCAT* | Phosphatidylcholine-sterol acyltransferase | -0.09 (0.25) | 0.05 (0.29) | 0.05 (0.26) | 0.06 (0.37) | -0.09 (0.21) |
| *LEG1* | Protein LEG1 homolog | -0.04 (0.35) | -0.05 (0.41) | 0.15 (0.5) | 0.1 (0.48) | -0.16 (0.37) |
| *LGALS3BP* | Galectin-3-binding protein | 0.09 (0.51) | 0.08 (0.45) | -0.03 (0.38) | -0.02 (0.42) | -0.14 (0.36) |
| *LMOD1* | Leiomodin-1 | -0.06 (0.66) | 0.46 (1.53) | 0.05 (1.42) | 1.02 (2.2) | 0.67 (1.48) |
| *LPA* | Apolipoprotein(a) | -0.06 (0.42) | 0.02 (0.37) | 0.08 (0.55) | 0.07 (0.54) | -0.03 (0.38) |
| *LRG1* | Leucine-rich alpha-2-glycoprotein | -0.11 (0.26) | 0.21 (0.39) | 0.12 (0.35) | 0.06 (0.49) | -0.05 (0.24) |
| *LRIG3* | Leucine-rich repeats and immunoglobulin-like domains protein 3 | 0.04 (0.52) | 0.14 (1.05) | 0.2 (0.83) | -0.15 (0.46) | -0.02 (0.8) |
| *LYVE1* | Lymphatic vessel endothelial hyaluronic acid receptor 1 | 0.38 (0.87) | 0.21 (1.08) | 0 (0.79) | -0.38 (0.92) | -0.09 (0.74) |
| *LZTFL1* | Leucine zipper transcription factor-like protein 1 | 0 (0.28) | 0.13 (0.31) | 0.15 (0.31) | 0.01 (0.36) | -0.05 (0.24) |
| *MARS1* | Methionine--tRNA ligase, cytoplasmic | 0.24 (0.78) | -0.11 (0.49) | 0.03 (0.77) | -0.15 (0.83) | 0.17 (0.75) |
| *MBL2* | Mannose-binding protein C | 0.11 (0.42) | 0.01 (0.39) | 0.09 (0.5) | 0.07 (0.65) | -0.09 (0.34) |
| *MCEMP1* | Mast cell-expressed membrane protein 1 | 0.31 (0.64) | 0.12 (0.61) | 0.14 (0.61) | -0.1 (0.48) | -0.14 (0.52) |
| *MDH1* | Malate dehydrogenase, cytoplasmic | -0.74 (1.81) | 0.12 (1.79) | -0.33 (1.6) | -0.16 (1.99) | -0.15 (2.02) |
| *MENT* | Protein MENT | -0.03 (0.56) | -0.15 (0.41) | -0.01 (0.46) | 0.02 (0.64) | -0.07 (0.54) |
| *MFAP4* | Microfibril-associated glycoprotein 4 | 0.03 (0.28) | 0.11 (0.28) | 0.1 (0.32) | -0.01 (0.32) | -0.01 (0.28) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *MKI67* | Proliferation marker protein Ki-67 | 0.03 (0.32) | 0.06 (0.4) | 0.03 (0.37) | 0.04 (0.37) | -0.07 (0.29) |
| *MOCS2* | Molybdopterin synthase catalytic subunit | 0.17 (0.53) | 0.26 (0.67) | 0.09 (0.77) | -0.08 (0.65) | -0.03 (0.54) |
| *MRC1* | Macrophage mannose receptor 1 | 0.26 (0.57) | 0.2 (0.7) | 0.1 (0.57) | 0.05 (0.69) | -0.11 (0.6) |
| *MRPS16* | 28S ribosomal protein S16, mitochondrial | -0.2 (1.14) | 0.3 (1.01) | -0.31 (1.23) | -0.06 (1.31) | -0.45 (1.29) |
| *MST1* | Hepatocyte growth factor-like protein | 0.24 (0.97) | 0.55 (0.8) | 0.11 (1.01) | 0.22 (1.53) | -0.06 (1.08) |
| *MTDH* | Protein LYRIC | 0.05 (0.13) | 0.04 (0.09) | 0.04 (0.14) | 0 (0.07) | 0 (0.08) |
| *MXRA8* | Matrix remodeling-associated protein 8 | -0.08 (0.32) | 0.05 (0.21) | 0.06 (0.31) | -0.02 (0.24) | -0.06 (0.17) |
| *MYOM3* | Myomesin-3 | 0 (0.34) | 0.02 (0.41) | -0.04 (0.37) | -0.05 (0.45) | -0.05 (0.31) |
| *NAGA* | Alpha-N-acetylgalactosaminidase | 0.21 (0.92) | -0.28 (0.68) | -0.12 (1) | -0.11 (1.06) | 0.16 (0.72) |
| *NAGPA* | N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase | 0.16 (0.32) | 0.34 (0.59) | 0.1 (0.57) | -0.13 (0.49) | -0.09 (0.6) |
| *NDUFA5* | NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 | 0.08 (0.34) | 0.1 (0.37) | 0.21 (0.43) | 0.02 (0.42) | -0.1 (0.26) |
| *NECTIN1* | Nectin-1 | 0.07 (0.35) | 0.02 (0.32) | 0.04 (0.4) | 0.06 (0.33) | 0.09 (0.48) |
| *NEDD4L* | E3 ubiquitin-protein ligase NEDD4-like | -0.12 (0.58) | -0.03 (0.42) | -0.02 (0.44) | -0.16 (0.59) | -0.21 (0.99) |
| *NEDD9* | Enhancer of filamentation 1 | 0.25 (0.62) | 0.17 (0.67) | -0.08 (0.84) | -0.19 (1.09) | 0.02 (0.62) |
| *NEXN* | Nexilin | 0.01 (0.42) | 0.16 (0.41) | -0.04 (0.46) | -0.07 (0.66) | -0.01 (0.39) |
| *NFAT5* | Nuclear factor of activated T-cells 5 | 0.08 (0.79) | 0.32 (1.04) | 0.37 (1.13) | 0.11 (1.08) | 0.05 (0.98) |
| *NHLRC3* | NHL repeat-containing protein 3 | -0.14 (0.72) | 0.01 (0.43) | 0.06 (0.48) | 0.25 (0.8) | -0.11 (0.52) |
| *NME1* | Nucleoside diphosphate kinase A | -0.04 (0.28) | 0.07 (0.27) | 0.07 (0.33) | 0.06 (0.4) | -0.12 (0.27) |
| *NPHS1* | Nephrin | 0.12 (0.37) | 0.04 (0.31) | 0.03 (0.39) | -0.04 (0.42) | -0.07 (0.38) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *NPHS2* | Podocin | 0.25 (0.85) | -0.09 (0.68) | 0.2 (0.7) | 0.21 (0.79) | 0.22 (0.86) |
| *NRGN* | Neurogranin | 0.22 (0.56) | 0.26 (0.61) | 0.04 (0.69) | -0.21 (0.69) | -0.05 (0.65) |
| *NUMB* | Protein numb homolog | -0.19 (0.83) | -0.07 (0.57) | 0.09 (0.67) | -0.02 (0.85) | 0.09 (0.74) |
| *NXPH3* | Neurexophilin-3 | 0.19 (0.39) | 0.14 (0.43) | 0 (0.29) | 0.11 (0.39) | -0.03 (0.46) |
| *OLFM4* | Olfactomedin-4 | 0.19 (0.75) | 0.32 (1.07) | -0.16 (0.96) | -0.24 (1.02) | -0.11 (1.01) |
| *ORM1* | Alpha-1-acid glycoprotein 1 | -0.04 (0.38) | -0.01 (0.3) | 0.09 (0.26) | 0.02 (0.37) | -0.13 (0.27) |
| *PALLD* | Palladin | 0.1 (0.31) | -0.01 (0.22) | 0.04 (0.21) | 0.06 (0.48) | 0.05 (0.39) |
| *PAXX* | Protein PAXX | 0.09 (0.5) | -0.16 (0.51) | -0.1 (0.45) | 0 (0.42) | -0.04 (0.48) |
| *PCBD1* | Pterin-4-alpha-carbinolamine dehydratase | 0.24 (1.45) | 0.36 (2.17) | 0.18 (1.56) | 0.34 (1.29) | 0.49 (1.5) |
| *PDE5A* | cGMP-specific 3',5'-cyclic phosphodiesterase | 0.2 (0.42) | 0.38 (0.79) | 0.12 (0.69) | -0.05 (0.56) | 0.02 (0.54) |
| *PDIA3* | Protein disulfide-isomerase A3 | 0.09 (0.71) | 0.27 (1.05) | 0.03 (1) | -0.25 (1.06) | -0.02 (0.76) |
| *PDZK1* | Na(+)/H(+) exchange regulatory cofactor NHE-RF3 | 0.49 (0.67) | 0 (0.43) | -0.19 (0.52) | 0.08 (0.64) | -0.03 (0.47) |
| *PENK* | Proenkephalin-A | -0.39 (1.7) | 0.14 (1.51) | 0.11 (1.55) | 0.35 (1.79) | -0.49 (1.89) |
| *PEPD* | Xaa-Pro dipeptidase | -0.3 (0.58) | -0.07 (0.61) | 0.12 (0.52) | 0.04 (0.47) | -0.08 (0.56) |
| *PER3* | Period circadian protein homolog 3 | 0.1 (0.45) | -0.05 (0.41) | 0.1 (0.9) | 0.06 (0.7) | 0.1 (0.76) |
| *PF4* | Platelet factor 4 | 0.2 (0.49) | 0.11 (0.55) | 0.11 (0.49) | -0.11 (0.43) | -0.03 (0.39) |
| *PGA4* | Pepsin A-4 | 0.19 (0.52) | 0.15 (0.36) | 0.17 (0.65) | -0.02 (0.6) | -0.02 (0.33) |
| *PGLYRP2* | N-acetylmuramoyl-L-alanine amidase | 0.58 (0.87) | 0.48 (1.32) | 0.13 (1.14) | -0.05 (1.07) | -0.04 (0.95) |
| *PGR* | Progesterone receptor | 0.03 (0.4) | 0.23 (0.64) | 0.04 (0.37) | 0.03 (0.5) | -0.1 (0.35) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *PHYKPL* | 5-phosphohydroxy-L-lysine phospho-lyase | 0.03 (0.49) | -0.05 (0.44) | 0.22 (0.7) | 0.17 (0.65) | -0.17 (0.39) |
| *PI16* | Peptidase inhibitor 16 | 0.89 (0.85) | 0.06 (0.65) | -0.13 (0.62) | 0.18 (1.02) | 0.06 (0.69) |
| *PIKFYVE* | 1-phosphatidylinositol 3-phosphate 5-kinase | 0.03 (0.24) | 0.13 (0.39) | -0.08 (0.47) | -0.05 (0.45) | -0.05 (0.33) |
| *PINLYP* | phospholipase A2 inhibitor and Ly6/PLAUR domain-containing protein | -0.19 (2.15) | 0.26 (1.56) | -0.16 (2.08) | -0.73 (2.3) | -0.09 (1.66) |
| *PLCB1* | 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1 | -0.2 (0.68) | 0 (0.58) | 0.1 (0.44) | -0.09 (0.54) | -0.17 (0.49) |
| *PLG* | Plasminogen | -0.27 (0.45) | 0.3 (0.74) | 0.14 (0.6) | 0.09 (0.63) | -0.07 (0.41) |
| *PNLIP* | Pancreatic triacylglycerol lipase | -0.14 (0.45) | 0.06 (0.32) | 0.01 (0.44) | -0.09 (0.48) | -0.15 (0.36) |
| *POF1B* | Protein POF1B | 0.07 (0.94) | 0.31 (1.34) | 0.35 (1.28) | 0.84 (2.09) | 0.55 (1.21) |
| *POLR2A* | DNA-directed RNA polymerase II subunit RPB1 | -0.06 (0.37) | 0.07 (0.58) | 0.04 (0.44) | -0.07 (0.5) | -0.09 (0.41) |
| *PON1* | Serum paraoxonase/arylesterase 1 | -0.03 (0.34) | 0.13 (0.4) | -0.06 (0.47) | -0.21 (0.55) | -0.06 (0.4) |
| *PPBP* | Platelet basic protein | 0.22 (0.65) | 0.29 (0.74) | 0.07 (0.86) | -0.08 (0.91) | -0.1 (0.53) |
| *PPL* | Periplakin | 0.03 (0.85) | -0.01 (0.62) | 0.04 (0.66) | 0.07 (0.71) | -0.07 (0.57) |
| *PPM1B* | Protein phosphatase 1B | 0.02 (0.31) | -0.03 (0.3) | -0.01 (0.43) | 0.18 (0.62) | 0.05 (0.5) |
| *PRDX2* | Peroxiredoxin-2 | -0.06 (0.26) | 0.09 (0.26) | 0.09 (0.36) | 0.08 (0.34) | -0.08 (0.2) |
| *PRKG1* | cGMP-dependent protein kinase 1 | -0.08 (0.42) | -0.21 (0.72) | 0.17 (0.7) | -0.05 (0.67) | 0.04 (0.6) |
| *PROS1* | Vitamin K-dependent protein S | 0.12 (0.51) | -0.12 (0.61) | -0.01 (0.63) | -0.07 (0.56) | 0.13 (0.57) |
| *PRR4* | Proline-rich protein 4 | 0.18 (0.67) | -0.16 (0.75) | -0.09 (0.85) | 0.29 (1.06) | 0.15 (1.22) |
| *PRR5* | Proline-rich protein 5 | -0.09 (0.27) | 0.03 (0.16) | 0.07 (0.2) | 0 (0.25) | -0.05 (0.29) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *PRSS22* | Brain-specific serine protease 4 | -0.23 (0.9) | 0.03 (0.38) | 0.14 (0.37) | -0.06 (0.43) | -0.12 (0.39) |
| *PSMG4* | Proteasome assembly chaperone 4 | 0.2 (0.42) | 0.1 (0.27) | 0.04 (0.56) | -0.08 (0.33) | -0.06 (0.36) |
| *PSTPIP2* | Proline-serine-threonine phosphatase-interacting protein 2 | 0.14 (1.51) | 0.28 (1.16) | 0.05 (1.19) | 0.47 (1.95) | 0.04 (1.03) |
| *PTGES2* | Prostaglandin E synthase 2 | 0.23 (0.95) | 0.35 (0.88) | 0.17 (0.96) | -0.07 (0.65) | 0.15 (0.63) |
| *PTP4A3* | Protein tyrosine phosphatase type IVA 3 | 0.35 (1.17) | 0.39 (1.41) | 0.04 (1.16) | 0.28 (1.42) | -0.37 (1.64) |
| *PTPN9* | Tyrosine-protein phosphatase non-receptor type 9 | -0.13 (0.26) | 0.09 (0.28) | 0.13 (0.31) | 0.03 (0.39) | -0.07 (0.25) |
| *PTRHD1* | Putative peptidyl-tRNA hydrolase PTRHD1 | 0.27 (0.82) | -0.03 (0.75) | -0.12 (0.88) | -0.2 (0.76) | 0.24 (0.93) |
| *PZP* | Pregnancy zone protein | 0.38 (1) | 0.3 (0.83) | 0.11 (0.88) | -0.01 (0.71) | 0.08 (0.65) |
| *QSOX1* | Sulfhydryl oxidase 1 | 0.12 (0.35) | 0.11 (0.86) | -0.03 (0.52) | -0.29 (0.67) | 0 (0.27) |
| *RABEP1* | Rab GTPase-binding effector protein 1 | 0.6 (1.39) | 0.58 (1.41) | 0.07 (1.43) | -0.23 (1.13) | 0.28 (1.06) |
| *RALB* | Ras-related protein Ral-B | 0.54 (0.92) | 0.52 (1.14) | 0.04 (0.95) | 0.05 (1.17) | -0.15 (1.02) |
| *RAP1A* | Ras-related protein Rap-1A | 0.08 (0.55) | -0.09 (0.73) | -0.06 (0.6) | 0.1 (0.73) | -0.11 (0.81) |
| *RBPMS* | RNA-binding protein with multiple splicing | 0.12 (0.75) | 0.14 (0.52) | 0.15 (0.64) | 0.16 (0.77) | -0.2 (0.52) |
| *RELB* | Transcription factor RelB | 0.29 (0.71) | -0.09 (0.66) | -0.06 (0.62) | 0.14 (0.95) | 0.1 (0.81) |
| *REPS1* | RalBP1-associated Eps domain-containing protein 1 | 0.46 (0.81) | 0.53 (0.89) | -0.01 (1.18) | -0.37 (1.1) | 0 (0.71) |
| *RICTOR* | Rapamycin-insensitive companion of mTOR | 0.31 (1.23) | -0.22 (1.02) | -0.01 (1.06) | -0.02 (1.12) | 0.27 (1) |
| *RIDA* | 2-iminobutanoate/2-iminopropanoate deaminase | -0.16 (0.31) | -0.04 (0.29) | 0.09 (0.23) | -0.04 (0.33) | -0.11 (0.25) |
| *RLN2* | Prorelaxin H2 | 0.44 (0.73) | 0.44 (0.8) | 0.11 (0.79) | 0.1 (0.84) | -0.06 (0.89) |

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| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *RNASE1* | Ribonuclease pancreatic | 0.1 (0.94) | 0.3 (0.84) | 0.08 (0.62) | 0.23 (0.89) | -0.19 (1.07) |
| *RNASE4* | Ribonuclease 4 | 0.06 (0.75) | 0.13 (1.29) | 0.36 (1.55) | 0.02 (1.83) | 0.01 (1.94) |
| *RNASE6* | Ribonuclease K6 | 0.54 (1.12) | 0.47 (1.46) | -0.04 (1.18) | 0.21 (1.29) | 0.14 (1.75) |
| *RNF168* | E3 ubiquitin-protein ligase RNF168 | 0.55 (1.62) | 0.08 (2.26) | 0.56 (1.34) | -0.03 (2.18) | 0.08 (1.78) |
| *RNF31* | E3 ubiquitin-protein ligase RNF31 | 0.2 (0.69) | 0.29 (1.45) | 0.07 (0.82) | -0.12 (0.72) | 0.01 (0.68) |
| *RPA2* | Replication protein A 32 kDa subunit | 0.1 (0.75) | 0.02 (0.57) | 0.24 (0.82) | 0.12 (0.68) | 0.22 (0.89) |
| *S100A13* | Protein S100-A13 | 0.19 (0.72) | 0.31 (0.52) | 0.25 (0.84) | 0.15 (0.48) | -0.19 (0.44) |
| *SAA4* | Serum amyloid A-4 protein | -0.12 (0.55) | 0.73 (1.93) | 0.4 (1.21) | 0.07 (0.66) | -0.15 (0.84) |
| *SCGB3A1* | Secretoglobin family 3A member 1 | -0.05 (0.21) | 0.1 (0.24) | 0.1 (0.23) | 0.04 (0.25) | -0.09 (0.16) |
| *SCRIB* | Protein scribble homolog | -0.14 (0.27) | 0.07 (0.33) | 0.12 (0.37) | 0.09 (0.36) | -0.1 (0.24) |
| *SDK2* | Protein sidekick-2 | -0.01 (0.31) | 0.08 (0.27) | 0.12 (0.33) | 0.13 (0.34) | -0.06 (0.21) |
| *SELENOP* | Selenoprotein P | -0.44 (0.99) | -0.15 (0.78) | 0.21 (0.98) | 0.3 (1.35) | -0.13 (0.97) |
| *SELL* | L-selectin | -0.27 (1.13) | 0.16 (1.08) | -0.03 (0.95) | 0.23 (1.31) | 0.02 (1.01) |
| *SEMA3G* | Semaphorin-3G | -0.02 (0.47) | -0.05 (0.55) | 0 (0.6) | 0.02 (0.81) | 0.14 (0.73) |
| *SEMA6C* | Semaphorin-6C | -0.05 (0.37) | 0.13 (0.4) | -0.07 (0.44) | -0.12 (0.51) | 0 (0.38) |
| *SERPINA1* | Alpha-1-antitrypsin | -0.06 (0.16) | 0 (0.18) | 0.04 (0.26) | -0.05 (0.24) | 0.05 (0.2) |
| *SERPINA3* | Alpha-1-antichymotrypsin | -0.14 (0.5) | -0.02 (0.24) | 0.05 (0.31) | 0 (0.37) | 0.01 (0.37) |
| *SERPINA4* | Kallistatin | 0.38 (0.77) | 0.16 (0.64) | 0.05 (0.64) | -0.04 (0.55) | -0.07 (0.76) |
| *SERPINA5* | Plasma serine protease inhibitor | -0.04 (0.42) | 0.03 (0.38) | 0.01 (0.4) | -0.06 (0.64) | -0.1 (0.44) |
| *SERPINA6* | Corticosteroid-binding globulin | -0.04 (0.37) | -0.02 (0.52) | 0.04 (0.37) | -0.03 (0.49) | -0.12 (0.37) |

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC). (*continued*)

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *SERPINA7* | Thyroxine-binding globulin | 0.02 (0.43) | -0.06 (0.38) | 0.03 (0.35) | 0 (0.37) | -0.08 (0.33) |
| *SERPINC1* | Antithrombin-III | 0.03 (0.43) | 0.03 (0.29) | 0.01 (0.4) | -0.05 (0.35) | -0.14 (0.38) |
| *SERPIND1* | Heparin cofactor 2 | -0.16 (0.33) | 0.09 (0.35) | 0.04 (0.47) | 0.09 (0.45) | -0.11 (0.4) |
| *SERPINF1* | Pigment epithelium-derived factor | -0.03 (0.24) | -0.01 (0.2) | 0.03 (0.24) | 0 (0.24) | -0.02 (0.17) |
| *SERPINF2* | Alpha-2-antiplasmin | -0.02 (0.32) | 0 (0.24) | 0.07 (0.29) | 0.01 (0.36) | -0.04 (0.33) |
| *SERPING1* | Plasma protease C1 inhibitor | -0.19 (0.36) | 0.13 (0.33) | 0.13 (0.28) | 0.06 (0.36) | -0.14 (0.27) |
| *SERPINI1* | Neuroserpin | -0.25 (0.41) | 0.1 (0.39) | 0.14 (0.37) | 0.07 (0.45) | -0.13 (0.3) |
| *SFRP4* | Secreted frizzled-related protein 4 | 0.08 (0.41) | 0.04 (0.26) | 0 (0.34) | 0.07 (0.53) | -0.04 (0.29) |
| *SHBG* | Sex hormone-binding globulin | 0 (0.37) | -0.05 (0.28) | -0.03 (0.63) | 0.01 (0.27) | -0.1 (0.3) |
| *SHH* | Sonic hedgehog protein | -0.11 (0.25) | 0.08 (0.29) | 0.1 (0.32) | 0.04 (0.3) | -0.07 (0.2) |
| *SIRT1* | NAD-dependent protein deacetylase sirtuin-1 | -0.11 (0.32) | -0.01 (0.4) | 0.12 (0.41) | -0.03 (0.44) | -0.1 (0.32) |
| *SLC9A3R1* | Na(+)/H(+) exchange regulatory cofactor NHE-RF1 | -0.19 (0.28) | 0.14 (0.32) | 0.16 (0.28) | -0.03 (0.28) | -0.17 (0.23) |
| *SLITRK1* | SLIT and NTRK-like protein 1 | -0.07 (0.32) | 0.09 (0.33) | 0.1 (0.37) | 0.01 (0.4) | -0.04 (0.28) |
| *SMPD3* | Sphingomyelin phosphodiesterase 3 | 0.05 (0.32) | -0.03 (0.2) | 0.11 (0.3) | 0.02 (0.36) | -0.04 (0.22) |
| *SNCA* | Alpha-synuclein | 0.16 (0.47) | -0.04 (0.35) | 0.04 (0.57) | 0.08 (0.6) | -0.04 (0.37) |
| *SNX15* | Sorting nexin-15 | 0.03 (0.34) | 0.09 (0.41) | -0.14 (0.54) | -0.27 (0.58) | -0.11 (0.45) |
| *SOD3* | Extracellular superoxide dismutase | 0.38 (0.56) | -0.18 (0.6) | -0.42 (0.83) | -0.03 (0.58) | -0.04 (0.63) |
| *SPART* | Spartin | -0.16 (0.61) | 0.01 (0.48) | -0.03 (0.62) | -0.07 (0.57) | 0 (0.56) |
| *SPINK2* | Serine protease inhibitor Kazal-type 2 | 0 (0.4) | 0.03 (0.62) | -0.05 (0.66) | 0.35 (1.26) | 0.15 (0.62) |

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC). (*continued*)

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *SPRED2* | Sprouty-related, EVH1 domain-containing protein 2 | 0.35 (0.74) | 0.17 (0.79) | 0.01 (0.6) | 0.06 (0.95) | -0.11 (0.9) |
| *SSBP1* | Single-stranded DNA-binding protein, mitochondrial | 0.37 (0.78) | -0.08 (0.41) | -0.27 (0.62) | 0.05 (0.69) | 0.07 (0.58) |
| *ST13* | Hsc70-interacting protein | -0.04 (0.95) | -0.02 (0.94) | -0.35 (1) | -0.17 (1.21) | -0.04 (0.8) |
| *ST8SIA1* | Alpha-N-acetylneuraminide alpha-2,8-sialyltransferase | 0.61 (1.81) | 0.84 (1.58) | 0.1 (1.9) | -0.22 (1.84) | -0.29 (1.44) |
| *STAT2* | Signal transducer and activator of transcription 2 | 0.17 (0.67) | 0.46 (0.78) | 0.09 (0.85) | -0.05 (0.67) | -0.14 (0.68) |
| *STX5* | Syntaxin-5 | 0.38 (1.22) | 0.46 (1.15) | 0.06 (0.78) | 0.26 (1.13) | 0.2 (0.91) |
| *STX7* | Syntaxin-7 | 0.39 (0.88) | 0.38 (0.89) | 0.04 (0.78) | 0.14 (1.02) | -0.22 (1.07) |
| *SUMF1* | Formylglycine-generating enzyme | -0.05 (0.5) | 0.02 (0.45) | -0.01 (0.52) | -0.33 (0.69) | 0.06 (0.49) |
| *SUSD5* | Sushi domain-containing protein 5 | 0.03 (0.24) | 0.14 (0.47) | 0 (0.33) | 0.1 (0.63) | -0.02 (0.3) |
| *SYAP1* | Synapse-associated protein 1 | 0.32 (0.8) | 0.06 (0.81) | -0.04 (1.01) | -0.01 (0.96) | -0.03 (0.8) |
| *TBCA* | Tubulin-specific chaperone A | 0.36 (1.05) | 0.49 (1) | 0.12 (1.16) | -0.26 (1.07) | 0.05 (0.82) |
| *TCN1* | Transcobalamin-1 | 0.21 (0.49) | 0.11 (0.44) | -0.09 (0.59) | 0.24 (1) | 0.15 (0.43) |
| *TERF1* | Telomeric repeat-binding factor 1 | 0.39 (0.97) | 0.36 (1.09) | 0.19 (1.01) | -0.31 (0.92) | 0 (0.74) |
| *TF* | Serotransferrin | 0.18 (0.64) | 0.06 (0.87) | 0.13 (0.92) | -0.08 (0.85) | 0.15 (0.71) |
| *TGFBR1* | TGF-beta receptor type-1 | 0.14 (0.63) | 0.25 (0.7) | 0.04 (0.7) | -0.03 (0.91) | -0.26 (0.72) |
| *TGOLN2* | Trans-Golgi network integral membrane protein 2 | 0.19 (1.07) | 0.55 (1.47) | 0.15 (1.7) | 0.3 (1.16) | 0.05 (1.17) |
| *THSD1* | Thrombospondin type-1 domain-containing protein 1 | 0 (0.24) | 0.11 (0.34) | 0.04 (0.37) | -0.11 (0.48) | 0.01 (0.38) |

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC). (*continued*)

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *TLR1* | Toll-like receptor 1 | 0.26 (0.57) | 0.22 (0.55) | 0.03 (0.47) | 0.16 (0.58) | -0.06 (0.63) |
| *TLR4* | Toll-like receptor 4 | 0.5 (1.17) | 0.63 (1.23) | 0.15 (1.35) | -0.07 (1.08) | -0.04 (1.09) |
| *TNFAIP8L2* | Tumor necrosis factor alpha-induced protein 8-like protein 2 | 0.03 (0.48) | -0.26 (0.38) | 0.12 (0.52) | -0.03 (0.44) | 0 (0.34) |
| *TNFRSF17* | Tumor necrosis factor receptor superfamily member 17 | 0 (0.64) | 0.12 (0.41) | 0.11 (0.48) | -0.08 (0.52) | 0.03 (0.39) |
| *TNFSF9* | Tumor necrosis factor ligand superfamily member 9 | -0.12 (0.31) | 0.2 (0.31) | 0.21 (0.44) | 0 (0.41) | -0.17 (0.28) |
| *TOP2B* | DNA topoisomerase 2-beta | 0.08 (0.63) | 0.21 (0.48) | 0.04 (0.57) | -0.18 (0.58) | -0.09 (0.43) |
| *TP53BP1* | TP53-binding protein 1 | 0.09 (0.38) | 0.01 (0.28) | 0.03 (0.3) | -0.04 (0.32) | -0.08 (0.28) |
| *TP53I3* | Quinone oxidoreductase PIG3 | 0.22 (0.38) | 0.1 (0.34) | -0.07 (0.65) | 0 (0.69) | -0.09 (0.48) |
| *TPD52L2* | Tumor protein D54 | -0.09 (0.55) | -0.04 (0.51) | 0.1 (0.41) | 0 (0.54) | -0.03 (0.42) |
| *TPSG1* | Tryptase gamma | 0.08 (0.31) | -0.1 (0.42) | 0 (0.36) | -0.08 (0.38) | -0.06 (0.46) |
| *TRAF3* | TNF receptor-associated factor 3, Isoform 2 | 0.12 (0.59) | 0.28 (0.5) | 0.1 (0.56) | -0.07 (0.58) | 0.05 (0.51) |
| *TREML1* | Trem-like transcript 1 protein | 0.22 (0.43) | -0.08 (0.44) | -0.05 (0.53) | 0.06 (0.42) | -0.1 (0.39) |
| *TSC1* | Hamartin | 0.33 (1.28) | -0.3 (0.62) | 0.15 (1.22) | -0.04 (0.72) | -0.04 (0.62) |
| *TSPYL1* | Testis-specific Y-encoded-like protein 1 | -0.04 (1.07) | -0.07 (1.01) | -0.06 (1.21) | -0.36 (1.36) | 0 (0.88) |
| *TTR* | Transthyretin | 0.23 (0.71) | -0.15 (0.6) | -0.08 (0.72) | -0.04 (0.84) | 0.11 (0.64) |
| *TXN* | Thioredoxin | 0.19 (0.63) | 0.29 (0.53) | 0.07 (0.7) | -0.14 (0.55) | -0.02 (0.5) |
| *TYRP1* | 5,6-dihydroxyindole-2-carboxylic acid oxidase | 0.31 (0.85) | 0.37 (1.04) | 0.11 (0.89) | -0.02 (1.01) | -0.24 (1.01) |
| *UBE2Z* | Ubiquitin-conjugating enzyme E2 Z | -0.16 (0.66) | 0.06 (0.59) | 0.08 (0.66) | 0.13 (0.93) | 0.05 (0.75) |

**Supplemental Table 5.1** Full list of proteins measured in the Olink Explore 384 Inflammation II panel including the mean and standard deviation in normalized protein expression (NPX). The proteins were measured in 21 children with intermediate uveitis (IU), 53 children with panuveitis, 36 children with idiopathic chronic anterior uveitis (iCAU), 44 children with juvenile idiopathic arthritis associated uveitis (JIA-U), and 22 pediatric controls (PC). (*continued*)

| Gene | Protein | PC mean (sd) | IU mean (sd) | Panuveitis mean (sd) | iCAU mean (sd) | JIA-U mean (sd) |
|---|---|---|---|---|---|---|
| *UBXN1* | UBX domain-containing protein 1 | 0.03 (0.65) | -0.05 (0.54) | 0.01 (0.64) | -0.14 (0.69) | 0.13 (0.57) |
| *UNC5D* | Netrin receptor UNC5D | -0.03 (0.99) | -0.04 (0.77) | -0.02 (0.67) | -0.02 (0.63) | -0.64 (0.94) |
| *UROD* | Uroporphyrinogen decarboxylase | 0.13 (0.94) | 0.29 (1.05) | -0.05 (1.3) | 0.15 (1.63) | 0.03 (1.17) |
| *VAMP8* | Vesicle-associated membrane protein 8 | 0.18 (0.54) | 0.13 (0.53) | 0.1 (0.51) | 0.01 (0.53) | -0.01 (0.5) |
| *VASP* | Vasodilator-stimulated phosphoprotein | -0.2 (0.4) | 0.18 (0.34) | 0.22 (0.41) | -0.01 (0.41) | -0.13 (0.3) |
| *VEGFB* | Vascular endothelial growth factor B | 0.34 (0.73) | 0.25 (0.61) | 0.07 (0.72) | -0.01 (0.58) | -0.01 (0.59) |
| *VNN1* | Pantetheinase | 0.2 (0.62) | 0.09 (0.65) | -0.06 (0.56) | -0.17 (0.66) | 0.18 (0.52) |
| *VTI1A* | Vesicle transport through interaction with t-SNAREs homolog 1A | 0.12 (0.61) | 0.23 (1.03) | 0.17 (0.76) | 0.37 (1.17) | 0.13 (0.96) |
| *WASL* | Neural Wiskott-Aldrich syndrome protein | 0.29 (0.8) | 0.44 (0.85) | 0.23 (0.99) | -0.04 (0.65) | -0.03 (0.59) |
| *XIAP* | E3 ubiquitin-protein ligase XIAP | 0.39 (0.8) | -0.05 (0.34) | -0.21 (0.55) | 0.09 (0.62) | 0.1 (0.57) |
| *YWHAQ* | 14-3-3 protein theta | 0.22 (0.99) | 0.53 (1.1) | 0.07 (1.28) | -0.25 (1.1) | -0.01 (0.77) |
| YY1 | Transcriptional repressor protein YY1 | 0.17 (0.76) | 0.3 (1) | 0.04 (0.87) | -0.04 (1) | -0.2 (0.92) |
| ZBP1 | Z-DNA-binding protein 1 | 0.27 (0.49) | 0.17 (0.56) | 0.03 (0.54) | 0.01 (0.59) | -0.01 (0.61) |
| ZNF174 | Zinc finger protein 174 | -0.02 (0.33) | 0.03 (0.36) | 0.14 (0.44) | -0.11 (0.5) | -0.09 (0.34) |

# CHAPTER 6

## Involvement of the systemic microcirculation in pediatric uveitis

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*Pediatric Rheumatol Online J. 2023; 21(1):109. doi: 10.1186/s12969-023-00896-7*

# ABSTRACT

## Background

Pediatric uveitis is a severe inflammatory ocular condition that can lead to sight-threatening complications and can negatively impact quality of life. The retinal microcirculation is often affected in intermediate uveitis and panuveitis. Here, we examined the extraocular (i.e., systemic) microcirculation in pediatric uveitis cases and healthy controls using nailfold capillaroscopy (NFC).

## Methods

We performed NFC in 119 children with noninfectious uveitis and 25 healthy pediatric controls, and assessed the following parameters: capillary density (number of capillaries/mm), dilated capillaries (apex > 20 µm), avascular area, the presence of microhemorrhages, and capillary morphology. Differences in NFC parameters between cases and controls were calculated using regression analysis after adjusting for age and sex.

## Results

The mean (± SD) age of the patient group was 13.7 (± 3) years, with 56% females; 46%, 18%, and 36% of cases presented as anterior uveitis, intermediate uveitis, and panuveitis, respectively, with an overall mean disease duration of 4.7 (± 4.0) years. Compared to the control group, the pediatric uveitis cases had a significantly higher number of dilated capillaries/mm and a higher prevalence of ramified capillaries. Moreover, compared to the control group the intermediate uveitis cases had a significantly higher number of dilated capillaries, whereas the anterior uveitis cases had a lower capillary density and a higher prevalence of ramified capillaries.

## Conclusions

Children with uveitis without systemic disease can present with changes in systemic microcirculation. These changes vary amongst the subtypes of uveitis.

## BACKGROUND

Uveitis is a complex, potentially sight-threatening ocular condition characterized by inflammation of the uvea and nearby structures involving the vessels in the retina and sclera.[1,2] Vessels in the anterior uveal tract may be affected as well, visible as anterior chamber flare caused by the breakdown of the blood-aqueous barrier due to the inflammatory process. Pediatric uveitis can develop in conjunction with a systemic condition, of which juvenile idiopathic arthritis (JIA) is the most common and best-studied. In up to 60% of cases, the etiology remains unknown, and these cases are considered an isolated idiopathic inflammatory eye condition.[1,3–6] Inflammatory involvement of the ocular vasculature is common in patients with intermediate uveitis or panuveitis and can include retinal vasculitis, diffuse retinal capillary leakage, and/or macular edema.[7–10] However, whether changes in the extraocular vascular and/or microcirculation are involved in pediatric patients with uveitis is currently unknown.



Nailfold capillaroscopy (NFC) is a noninvasive diagnostic technique used to evaluate the microcirculation in the nailfold. This technique is commonly used in the medical fields of rheumatology and dermatology, revealing various abnormal patterns associated with diseases such as systemic sclerosis and systemic lupus erythematosus (SLE).[11–14] Ocular manifestations, including changes in the retinal or choroidal microcirculation, are described in these rheumatic diseases. More recently, NFC has also been used to detect microvascular abnormalities in ocular diseases, particularly glaucoma.[15–17] Moreover, Chen *et al.* used NFC in adults with uveitis and found abnormalities in the systemic microcirculation that were correlated with peripheral retinal leakage.[18] Because NFC is noninvasive, easy to perform, and relatively rapid, it is highly suitable for use in children.[14–17] To date, however, the use of NFC in children with uveitis has not been described. Therefore, the primary aim of this study was to compare NFC findings between pediatric uveitis cases and healthy pediatric controls, and to compare findings between uveitis subtypes. Our results may provide new insights into the pathogenesis of this potentially sight-threatening pediatric condition, as well as new insights regarding the development and application of new diagnostic and/or prognostic biomarkers.

## METHODS

### Study population

The participants in this cross-sectional study were recruited between June 2020 and June 2022 at the Ophthalmology Department of the University Medical Center

Utrecht (UMCU), the Netherlands, a tertiary referral center. The pediatric control group consisted of children with strabismus and no evidence of a concomitant inflammatory condition. We excluded participants with any cardiovascular or other systemic disease not associated with uveitis, including type 2 diabetes mellitus and hypertension. We also excluded participants with infectious uveitis based on serology or ocular fluid analysis. All cases were diagnosed by a pediatric uveitis specialist at the UMCU Department of Ophthalmology according to the SUN criteria.[2] The presence of associated systemic diseases was assessed in accordance with current diagnostic criteria for pediatric uveitis, and all patients were assessed by pediatricians and/or pediatric rheumatologists.[19] The analytical screening performed prior to classifying uveitis as idiopathic is shown in **Supplemental data.** All participants and/or their legal guardian provided written informed consent before participating in this study. This study adhered to the tenets of the Declaration of Helsinki and was approved by the UMCU Medical Research Ethics Committee (protocol number: 20-317).

## Clinical data

Demographic data obtained from all participants included age at NFC assessment and sex; in addition, the following disease-specific information was collected for the patients with uveitis: date of uveitis diagnosis, uveitis anatomic subtype (anterior uveitis, intermediate uveitis, or panuveitis), uveitis disease duration, presence (and type, if applicable) of an associated systemic disease, and laboratory results of antinuclear antibody (ANA) testing. ANA positivity was defined as a titer of ≥ 1:160 detected on the HEp-2 indirect immunofluorescence assay on 2 occasions at least 3 months apart.[20] The following ophthalmological findings were reported: presence of anterior chamber flare of 1+ or higher, presence of cystoid macular edema (CME), papillitis, retinal vasculitis and/or retinal capillary leakage on fluorescein angiography (FA). CME was defined as the presence of macular thickening with cyst formation visible on macular OCT and/or FA.[21–23] Papillitis was defined as the presence of optic disc hyperfluorescence and/or leakage on FA, inflammatory optic disc swelling, and/or retinal nerve fiber layer thickness of >130 µm on OCT while excluding all other noninflammatory causes of optic disc swelling.[24] In order to monitor changes in NFC findings over time, a small prospective cohort consisting of 20 patients in original cohort of noninfectious uveitis cases underwent two additional NFC measurements 6 and 12 months after the first measurement. These 20 patients did not use any systemic medication the first NFC measurement.

## Nailfold capillaroscopic technique

A standardized NFC technique from the European Alliance of Associations for Rheumatology (EULAR) study group on Microcirculation in Rheumatic Diseases was used

for the examination and classification.[12,14,25] Prior to the NFC examination (CapillaryScope 200 Pro, Dino-Lite), each participant was acclimatized for at least 15 min at room temperature (20–24°C). The nailfold of all fingers, except for the thumbs, was examined after applying a drop of immersion oil on the nailfold bed to improve resolution. NFC was performed by a single observer, first at low (50x) magnification in order to determine the distribution of any obvious abnormalities and to obtain an overview of the nailfold area (1–2 images were obtained). Next, four consecutive images were obtained at 200 × magnification for assessing the detailed morphology of the capillaries. The images were analyzed at a later stage by the same observer who obtained the images. Three different second observers also analyzed images for the presence of hemorrhages and to determine the capillary morphology. The intraclass correlation coefficient for the assessment of hemorrhages and abnormal morphology was 0.57–0.88; all cases with a low correlation were discussed together until consensus was reached. During the analysis, the first and second observers were both blinded with respect to the participants' details. DinoCapture 2.0 software was used to analyze the images.[26]



The following NFC parameters were assessed at 200 × magnification:

- *Capillary density*, defined as the number of capillary loops per mm in the distal row using the "90° method", where a capillary loop was considered to be a distal loop if the apex of the capillary made an angle of ≥90° with the apex of its adjacent capillaries.[27]
- *Density of dilated capillaries*, defined as the number of dilated capillaries with an apical limb diameter of 20–50 µm per mm.[12,14]
- *Microhemorrhages* visible as hemosiderin deposits within the distal row of the nailfold, caused by the rupture of one or more capillaries.[12,14]
- *Avascular areas*, defined as the absence of capillaries in the distal row with a minimum of 200 µm between adjacent capillaries.[18]
- *Capillary morphology*, scored per capillary per image as normal, multiple crossings, tortuous, bushy, ramified, nonconvex, or bizarre shaped.[12,27] The definitions of each capillary morphology are shown in **Table 6.1**. Capillaries with busy, ramified, nonconvex, and bizarre morphologies were also classified as having an abnormal morphology.[12,14,25] The percentage of each morphology type was calculated by dividing the total number of capillaries of that morphology type by the total number of capillaries assessed per image.

**Table 6.1** Definitions of the various types of capillary morphologies[12,28–31]

| Capillary morphology | Definition |
|---|---|
| Normal | Capillaries with a hairpin shape or a once crossing shape, on the condition that the apex is convex. |
| Multiple crossings | The capillary limbs cross at least twice. |
| Tortuous | Curled capillary limb without crossing of the limb. |
| Ramified | Branched capillary limb, like a tree without leaves. |
| Bushy | Capillary loop with limbs originating from small and multiple buds instead of branches. |
| Nonconvex | The form of the apex is not curved like the exterior of a circle or sphere (convex). |
| Bizarre | Atypical morphology, distinct from the other described morphologies. |

Note: tortuous, ramified, bushy, and bizarre capillaries are considered "abnormal".

To obtain the NFC parameters at the participant level, mean values were calculated, except for the presence of microhemorrhages, in which the presence of a microhemorrhage in at least one image was considered positive for that participant. We also assessed the quality of the images and excluded any participant in which fewer than 25% of the images were deemed eligible for assessment.[14] **Figure 6.1** shows a representative NFC image of a pediatric control.

## Statistical analysis

All statistical analyses were performed using the R software package version 4.2.2. The intraclass correlation coefficient between observers was calculated for the assessment of capillary morphology. Descriptive statistics are used to report demographics and to describe the NFC parameters per group. Except where indicated otherwise, summary data are represented as the mean and standard deviation (for continuous variables) or percentage (for categorical variables). To compare NFC parameters between groups, we performed a multivariable regression analysis, adjusted for age and sex. Differences were considered significant at $P < 0.05$, and all tests were 2-tailed.

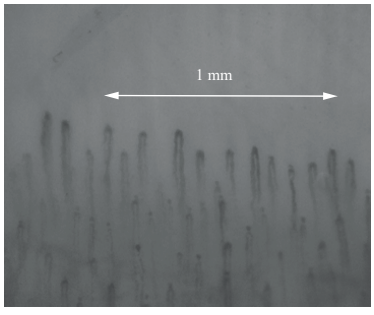


**Figure 6.1** Normal nailfold capillaroscopy image of a 17-year-old female control, with a capillary density of 10 capillaries per linear mm. Note the absence of dilated capillaries, hemorrhages, and avascular areas, with normal capillary morphology.

## RESULTS

Initially, a total of 154 patients and controls were examined with NFC, after which 10 participants (9 patients and 1 control) were excluded based on an insufficient number of assessable images. Thus, a total of 144 participants (119 patients and 25 controls) were included in our analysis. The demographics and clinical features of these groups are summarized in **Table 6.2**. Twelve of the 38 patients diagnosed with JIA-associated uveitis received systemic treatment for their uveitis. In the other patients systemic treatment was indicated for both JIA and uveitis.

**Table 6.2** Participant characteristics of patients with pediatric uveitis and pediatric controls

| Demographics | Uveitis | Pediatric controls | *P*-value |
|---|---|---|---|
| Number of subjects | 119 | 25 | |
| Male sex, *n* (%) | 52 (44) | 13 (52) | 0.54 |
| Mean age in years (SD) | 13.7 (3) | 9.1 (4) | **<0.001** |
| Type of uveitis, *n* (%) | | | |
| Anterior | 55 (46) | NA | |
| Intermediate | 21 (18) | | |
| Panuveitis | 43 (36) | | |
| Associated systemic disease, *n* (%) | | | |
| None | 62 (52) | NA | |
| JIA | 38 (32) | | |
| Presumed TINU syndrome | 13 (11) | | |
| Other[a] | 6 (5) | | |
| Disease duration of uveitis in years | 4.7 (4) | NA | |
| Type of JIA, *n* (%) | | | |
| Monoarthritis | 2 | NA | |
| Oligoarthritis | 28 | | |
| Polyarthritis | 8 | | |
| ANA seropositive, *n* (%) | 60 (50) | NA | |
| Systemic treatment, *n* (%) | | | |
| None | 25 (21) | 25 (100) | |
| Corticosteroids | 16 (13) | 0 | |
| Methotrexate | 45 (38) | 0 | |
| Mycophenolate mofetil | 45 (38) | 0 | |
| Adalimumab | 36 (30) | 0 | |
| Infliximab | 3 (3) | 0 | |
| Tocilizumab | 10 (8) | 0 | |
| Other[b] | 4 (3) | 0 | |

ANA = antinuclear antibody; DMARD = disease-modifying antirheumatic drug; JIA = juvenile idiopathic arthritis; NA = not applicable; SD = standard deviation; TINU = tubulo-interstitial nephritis and uveitis.
[a]Vogt-Koyanagi-Harada Disease (*n* = 3), psoriasis vulgaris (*n* = 2), or Blau syndrome (*n* = 1).
[b] Azathioprine (*n* = 1), Golimumab (*n* = 1), Leflunamide (*n* = 1), Tofacitinib (*n* = 1).

## Comparison between cases and controls

We found significant differences between the patient group and the control group with respect to the number of dilated capillaries per mm, the prevalence of ramified capillaries, and the mean percentage of ramified capillaries (defined as the number of ramified capillaries divided by the total number of capillaries). Analysis of patients without an associated systemic disease (*n* = 61) showed similar results (data not shown). No other NFC parameters differed significantly between the two groups, even when we combined the four abnormal morphologies (busy, ramified, nonconvex, and bizarre) into one category (**Table 6.3**).

**Table 6.3** Summary of nailfold capillaroscopy parameters in the noninfectious uveitis group and pediatric controls

| Nailfold capillaroscopic parameters | Uveitis, *n* = 119 | Pediatric controls, *n* = 25 | *P*–value[a] |
|---|---|---|---|
| Capillary density, mean (SD) | 7.2 (0.7) | 7.5 (0.7) | 0.05 |
| Capillary density <7/mm, *n* (%) | 50 (42) | 5 (20) | 0.07 |
| Dilated capillaries/mm, median (IQR) | 0.2 (0-0.4) | 0 (0-0.1) | **0.04** |
| Microhemorrhages, *n* (%) | 17 (17) | 8 (32) | 0.97 |
| Avascular areas, median (IQR) | 0.3 (0.2-0.4) | 0.2 (0.1-0.3) | 0.20 |
| **Capillary morphology**[b] | | | |
| Multiple crossings, mean % (SD) | 5 (4) | 2 (3) | 0.06 |
| Tortuous capillaries, mean % (SD) | 7 (6) | 6 (6) | 0.68 |
| Bushy capillaries, mean % (SD) | 1 (1) | 1 (1) | 0.23 |
| Ramified capillaries, mean % (SD) | 2 (2) | 1 (1) | **0.02** |
| Nonconvex, mean % (SD) | 3 (2) | 4 (2) | 0.16 |
| Bizarre capillaries, mean % (SD) | 2 (3) | 1 (2) | 0.17 |
| Abnormal morphology[c], mean % (SD) | 5 (4) | 5 (4) | 0.79 |

IQR = interquartile range; SD = standard deviation

[a] Calculated using a multivariate regression analysis adjusted for age at NFC examination and sex.

[b] The percentage of each morphology type was calculated by dividing the sum of the number of capillaries with that morphology type by the total number of capillaries assessed per image.

[c] Abnormal morphology includes bushy, ramified, nonconvex, and bizarre shaped capillaries.

## Uveitis subtypes

Next, we analyzed the NFC parameters in the pediatric controls and in each of the three specific uveitis subtypes (anterior uveitis, intermediate uveitis, and panuveitis); the results are summarized in **Figure 6.2**. We found that mean capillary density was significantly lower in the patients with anterior uveitis (7.0 ± 0.7) compared to the control group (7.5 ± 0.7, *P* = 0.008). Moreover, 54% of the patients with anterior uveitis (30/55 cases) had a capillary density <7/mm, compared to 20% (5/25) of the pediatric controls (*P* = 0.01). We also found that the patients with intermediate uveitis had a significantly higher number of dilated capillaries per mm compared to

the pediatric controls ($P < 0.001$). Finally, the mean percentage of ramified capillaries was higher in the patients with anterior uveitis and in the patients with panuveitis compared to the pediatric controls.

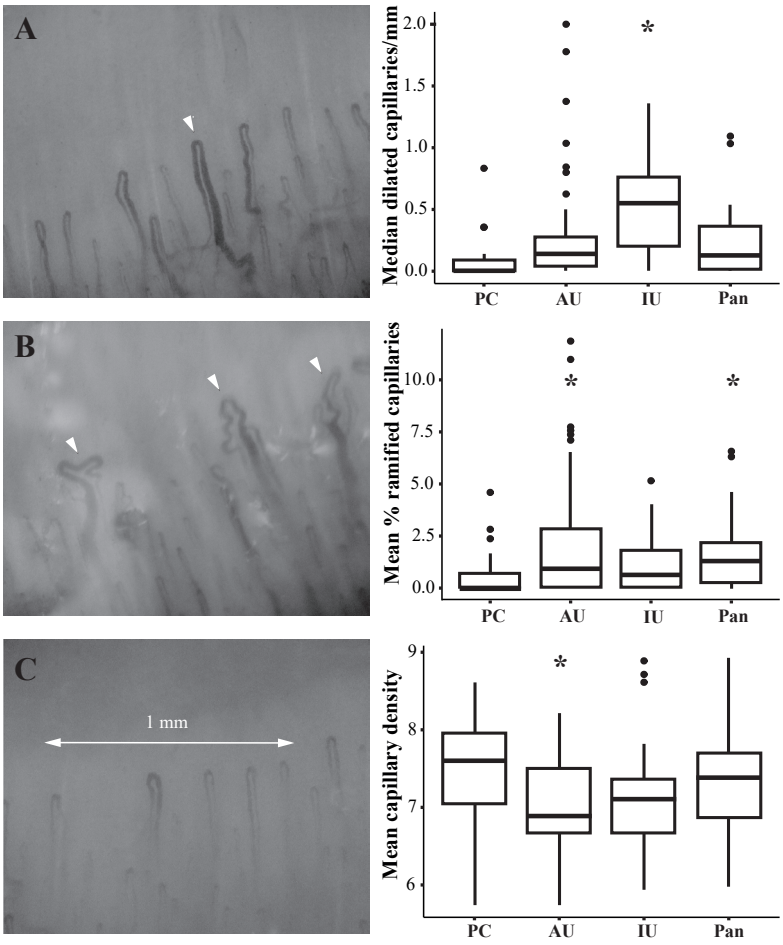


**Figure 6.2** Nailfold capillaroscopy findings for pediatric controls (PC; $n = 25$), anterior uveitis cases (AU, $n = 55$), intermediate uveitis cases (IU, $n = 21$), panuveitis cases (Pan, $n = 43$). **A.** NFC image of a 16-year-old boy diagnosed with panuveitis showing a dilated capillary (left, arrowhead) and boxplot summarizing the median number of dilations per linear mm in each subgroup (right). **B.** NFC image of the same 16-year-old boy diagnosed with panuveitis showing ramified capillaries (left, arrowheads) and boxplot summarizing the mean percentage of ramified capillaries in each subgroup (right). **C.** NFC image of a 16-year-old girl diagnosed with anterior uveitis showing low capillary density (< 7 capillaries per linear mm) (left) and boxplot summarizing mean capillary density in each subgroup (right). *$P < 0.05$ versus the control group, determined using linear regression analysis adjusted for age and sex.

We also found differences in certain NFC parameters when we compared the 55 patients with anterior uveitis with the intermediate uveitis and panuveitis patients combined ($n$ = 64). Specifically, the patients with anterior uveitis had a lower mean capillary density (7.0/mm vs. 7.3/mm, respectively, $P$ = 0.008), a higher percentage of abnormally shaped capillaries (6% vs. 4%, respectively, $P$ = 0.02), and a higher percentage of tortuous capillaries (7% vs. 4%, respectively, $P$ = 0.02); all other parameters were similar between these two subgroups.

In addition, we found no difference between the 38 patients with JIA-associated uveitis and the 16 patients with idiopathic chronic anterior uveitis (data not shown). When comparing the presence of anterior chamber flare in patients with anterior uveitis, we found a higher percentage of bushy capillaries in patients without the presence of flare compared to patients with anterior chamber flare ($n$ = 17, 0.9% vs. $n$ = 38, 0.3%; $P$ = 0.04). The other NFC-parameters were not significantly different. Also no difference was found within the different types of JIA.

Additionally, we compared ANA-positive patients ($n$ = 60) to ANA-negative patients ($n$ = 59), and found that ANA-positive patients had a significantly lower capillary density (7.0 vs. 7.3, respectively, $P$ = 0.02) and more microhemorrhages ($P$ = 0.04) compared to ANA-negative patients.

We also assessed the association between NFC parameters and inflammatory vascular involvement of the retina based on FA findings in 47 patients. We found that capillary density was significantly higher in the patients with papillitis (7.4/mm) compared to the patients without papillitis (7.1/mm, $P$ = 0.04); however, this difference was no longer significant after we corrected for uveitis subtype. In contrast, we found no significant association between CME, retinal vasculitis and/or retinal capillary leakage on FA and any of the NFC parameters.

Finally, we compared patients with a capillary density <7/mm ($n$ = 50) to patients with a capillary density ≥7/mm ($n$ = 69). We found that patients with a capillary density <7/mm had a longer duration of uveitis, and more patients were treated with biological agents for adequate disease control compared to patients with a capillary density ≥7/mm when adjusting for age, sex, and duration of uveitis. We did not found an association with disease activity, and use of medication.

## Follow-up NFC data

A total of 20 patients in the uveitis group underwent two follow-up NFC measurements 6 and 12 months after the first measurement. We found no significant difference in

NFC parameters between the first NFC measurement and either of the two follow-up measurements (data not shown). Interestingly, however, we found that the percentage of microhemorrhages decreased—albeit not significantly—between the initial NFC measurement (55%) and the 6-month and 12-month measurements (33% and 35%, respectively).

## DISCUSSION

In this study, we report significant differences in NFC parameters between children with noninfectious uveitis and pediatric controls, suggesting involvement of the systemic microcirculation in pediatric uveitis. Our results also suggest that the systemic microcirculation is affected even in idiopathic cases of uveitis that present without an identified systemic condition. Previous studies have shown that several inflammatory indicators such as the neutrophil/lymphocyte ratio, interleukins, and the platelet/lymphocyte ratio are elevated in the serum of patients with noninfectious uveitis .[32–43] These increased indicators are associated with both the activity and the severity of uveitis, likely implying activation of the systemic immune system (i.e., an inflammatory index). Our results support the notion that uveitis is not exclusively an intraocular inflammation but can also indicate systemic involvement. Although the long-term consequences of these changes in the microcirculation are currently unknown, our findings underscore the complexity of this potentially sight-threatening condition in children and provides new insights for developing diagnostic and prognostic biomarkers to monitor the microcirculation.

A previous cross-sectional case-control study in adults with uveitis found differences in NFC assessment, with a higher number of dilated capillaries and lower capillary density in the patient group.[44] Although we found no difference in capillary density between our entire pediatric uveitis group and the control group, we did find a significantly lower capillary density in the patients with anterior uveitis. This difference between studies might be due—at least in part—to differences between adult patients and pediatric patients and/or the slightly higher percentage of anterior uveitis patients in the previous study in adults (54%) [44] compared to our study (46%).

Recently, Melsens *et al.* reported that healthy children have a similar capillary density as adults and that the same cut-off value used for adults (≥7 capillaries per linear mm) can also be used in children.[14] Although not statistically significant, we found that 42% of the children with uveitis in our study had a capillary density <7/mm compared to 20% of pediatric controls; this difference may be attributed in part to the fact that

nearly half of the patients in our study had anterior uveitis, as anterior uveitis is often accompanied by a systemic disease such as JIA. However, the presence of JIA cannot fully explain this finding, as we found no difference between JIA-associated uveitis and idiopathic chronic anterior uveitis (data not shown). In addition, these two subtypes of uveitis are considered to be clinically identical, as they share genetic risk alleles and cannot be distinguished based solely on ophthalmological features.[45,46] Moreover, recent studies in children with JIA found no changes in NFC parameters between patients with JIA (but for whom the presence of uveitis was unknown) and controls, indicating that arthritis might not be the cause of the changes in the microcirculation.[14,47] Despite no association was found between lower capillary density and disease activity in this study, we do believe that a lower capillary density might be associated with a worse disease control. To reach an adequate disease control these patients needed the addition of a biological agent in contrary to patients with higher capillary density.

Interestingly, we found that the patients with intermediate uveitis had the highest number of dilated capillaries per mm. Intermediate uveitis is often accompanied by signs of inflammatory involvement of the retina such as intraocular perivasculitis, vasculitis, and periphlebitis. In contrast to other studies in adults [44,48], we found no apparent correlation between abnormalities in the retinal and nailfold microcirculation.

While vessels in the anterior uveal tract may potentially be affected especially in anterior uveitis, there is currently no practical method available to directly visualize them in clinical practice. However, the measurement of the anterior chamber flare could potentially serve as a clinical representation of this inflammatory process and the consequent breakdown of the blood-aqueous barrier as a result of it. We found no association between anterior chamber flare and NFC-parameters, with the exception of bushy capillaries. However, the found differences were very small, which might question the clinical relevance of this finding. The ciliary body might also be affected in anterior uveitis. However, it is difficult to investigate possible abnormalities regarding the vasculature with the current methods of examination.

Nailfold capillaroscopic changes are extensively described and investigated in several rheumatic diseases such as systemic sclerosis, SLE, and dermatomyositis.[11–14] Interestingly, in literature, ocular manifestations, including abnormalities in the microcirculation of the retina and choroid, are described in these rheumatic diseases.[49–55] Several of these studies use optical coherence tomography angiography (OCT-A) as a non-invasive tool to assess the retinal and choroidal microcirculation.[49,50,52,54,55] Jakhar *et al.* revealed that patients with systemic sclerosis with retinal disease have more severe

NFC changes.[56] Studies investigating a possible association between NFC changes and retinal/choroidal microcirculatory changes are currently lacking. Therefore, future research addressing this topic might provide valuable information regarding the interaction between the microcirculation of the nailfold and the retina/choroid.

One strength of our study is that we included a relatively large cohort of children with uveitis. Furthermore, we assessed the NFC images using a standardized protocol based on the international consensus definitions established by the EULAR Study Group on Microcirculation in Rheumatic Diseases [12,25]; these definitions were used recently in a standardized assessment of children.[14] In addition, the majority of the images was assessed by two observers who were blinded with respect to the participant's details.

Despite these strengths, our study also has some limitations that warrant discussion. First, the pediatric control group was not age-matched to the patient group, resulting in a significant difference in age between the two groups; however, we corrected for both age and sex in of our analyses. Second, data whether patients with JIA-associated uveitis were receiving immunosuppressive drugs primarily for arthritis or for uveitis was not always clearly available in this study. Third, our prospective study did not show any apparent change in NFC findings, even after one year; however, this may have been too short of a follow-up period to detect changes, as a previous study involving patients with systemic sclerosis found that the median time to progression of the NFC pattern (i.e., the time after which 50% of patients did progress) was nearly four times as long as our follow-up period.[57] Although not statistically significant, we did observe a decrease in microhemorrhages in our prospective study. Whether this is due to effects of treatment is currently unclear due to the small number of patients. The clinical repercussions of the findings in this study are currently unknown and are yet to be determined in future studies. Longitudinal studies with repeated NFC measurements for a longer follow-up period are therefore needed in order to determine whether NFC findings have prognostic value with respect to predicting the course of disease severity in noninfectious uveitis. Furthermore, it would be interesting to investigate whether NFC findings can be used to predict disease relapse, the need for additional systemic treatment, and/or treatment response in patients with pediatric uveitis. On the other hand, future studies investigating the underlying pathophysiology are also needed in order to identify biomarkers specific to uveitis-associated inflammation and/or neoangeogenesis. Such studies will likely provide valuable information regarding the underlying disease mechanisms and may provide simple prognostic indicators that can be used to guide the precision care of patients with noninfectious uveitis.

## CONCLUSIONS

Pediatric uveitis can present with changes in the systemic microcirculation, with specific differences based on the subtype of uveitis. Our results suggest that noninfectious pediatric uveitis is not always limited to intraocular inflammation, but may also include systemic inflammation. The changes observed in the systemic microcirculation do not appear to be correlated with retinal vascular inflammation involvement in our clinically heterogenous cohort. Thus, whether capillary changes reflect vascular involvement in specific uveitis subtypes—for example, intermediate uveitis—warrants further investigation. Nevertheless, our findings provide important new insights into this severe, potentially sight-threatening condition in children and provide input for designing both prospective studies and translational studies.

## ACKNOWLEDGEMENTS

This work was supported by funding from the Oogfonds and Dr. F.P. Fischer-Stichting, which contributed through UitZicht. The ODAS Stichting also funded the study. The funders did not play a role in the study design, collection, analysis, interpretation, or reporting of these data.

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# SUPPLEMENTAL DATA

## Screening for associated systemic diseases in pediatric uveitis patients

The performed tests for screening might be adjusted in individual cases based on clinical indication. The routine screening includes serology, urinary and virology testing, and chest-X-ray.

*Laboratory serology tests*

Sodium, potassium, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), complete blood count including blood differential, antinuclear antibody serology, human leukocyte antigen (HLA)-B27, angiotensin-converting enzyme (ACE), calcium, albumin.

*Urinary tests*

Beta2-mircoglobulin, creatinine, protein/creatinine ratio.

*Virology tests*

Treponema pallidum, QuantiFERON, human immunodeficiency virus (HIV).

*Chest-X-ray*

# CHAPTER 7

## Summary, general discussion and future perspectives

Childhood uveitis represents a rare group of ophthalmological inflammatory entities with a varied spectrum of clinical presentation and treatment. Physicians face several challenges in the management of this potentially vision-threatening disease. Research into pediatric uveitis has mainly focused on juvenile idiopathic arthritis-associated uveitis (JIA-U), with less information available for clinical management and molecular understanding of other specific subtypes. In this thesis, we studied a variety of aspects of pediatric uveitis beyond JIA-U with the overarching aim to improve the current diagnostic and therapeutic strategies for pediatric uveitis in general. These new insights may serve as a foundation for more personalized interventions and aid in the development of new approaches for treatment. This chapter summarizes the main findings of this thesis, puts them in a broader perspective and proposes future research opportunities.

## SUMMARY OF THE MAIN FINDINGS

### Clinical outcomes in patients with idiopathic chronic anterior uveitis



Previous studies on pediatric uveitis have primarily focused on children with juvenile idiopathic arthritis-associated uveitis (JIA-U), resulting in a limited insight of idiopathic chronic anterior uveitis cases (iCAU). To fill this gap, we performed a retrospective cohort study in **Chapter 2**. We examined the medical records of 48 children with iCAU and 48 age-matched children with JIA-U, and compared their disease course, visual outcome, ocular complications and treatment. The main finding of this study revealed that children with iCAU exhibit a more complicated disease course in comparison to those with JIA-U. Notably, a higher prevalence of posterior synechiae and a greater likelihood of cataract requiring surgery were observed in children with iCAU, both at the time of uveitis diagnosis and during follow-up. We suspect that this higher prevalence of complications might be related to a relatively longer period of under-treatment as a result of the usually later detection due to the asymptomatic nature of iCAU and to the absence of screening, as compared to JIA-U. To further investigate this, we performed a subgroup analysis of children with JIA diagnosed with uveitis prior to arthritis. This analysis confirmed this hypothesis and showed that JIA patients with an onset of uveitis prior to arthritis had baseline conditions similar to those of patients with iCAU.

Additionally, we identified younger age at diagnosis (≤8 years of age) as a risk factor for ocular complications within the iCAU cohort, which is in agreement with observations made previously in JIA-U. In contrast, however, male sex was not identified as a risk factor for iCAU, although male sex as a risk factor for JIA-U is not conclusive in all

studies either. Despite the differences in vision-threatening ocular complications between the two groups, long-term vision remained good and did not differ significantly between groups.

## Retinal nerve fiber layer thickness on OCT for diagnosing papillitis in pediatric uveitis

Papillitis is an inflammation of the optic nerve head resulting in swelling of the optic disc. Fluorescein angiography (FA) is considered as the gold standard for detecting papillitis in pediatric uveitis. However, this technique is relatively invasive and particularly challenging to perform in young children. In addition, fundoscopic identification of papillitis can be complicated by many factors. In **Chapter 1**, an alternative non-invasive imaging method, optical coherence tomography (OCT), specifically the measurement of the retinal nerve fiber layer thickness using OCT (OCT-RNFL), was proposed as a feasible technique for evaluating papillitis. To investigate the diagnostic value of OCT-RNFL in diagnosing papillitis in pediatric uveitis, a retrospective cohort study was conducted in **Chapter 3**.

Initially, we performed receiver operating characteristics (ROC) analysis to compare FA with OCT-RNFL in a subgroup of 93 children with different subtypes of pediatric uveitis. We determined an ideal cut-off value for OCT-RNFL thickness of 130 µm with relatively high sensitivity and specificity. By addition of ophthalmological data, including the presence of cystoid macular edema (CME), disease activity, and optic disc swelling on fundoscopy, as covariates/parameters into the regression model based on OCT-RNFL values significantly enhanced the model's ability to classify papillitis from non-papillitis cases with pediatric uveitis. Therefore, these readily available ophthalmological data should be taken into account to obtain a more accurate detection of papillitis.

Next, the established OCT-RNFL cut-off value of 130 µm was applied to our entire study cohort of 257 pediatric uveitis patients. Among them, slightly more than one-third of the patients had an OCT-RNFL thickness greater than 130 µm. As expected, the majority of these patients were diagnosed with intermediate and panuveitis. The most common visual field defect observed in patients with an OCT-RNFL thickness >130 µm was an enlarged blind spot. Papillitis serves as a clinical indicator reflecting uveitis activity. Consequently, the ability to monitor papillitis at regular intervals might improve our ability to adjust the treatment course minimizing the risk of permanent visual impairment. To conclude, RNFL thickness measured on OCT offers non-invasive, rapid, and easy-to-perform measurements in children, providing valuable and additional insights for disease monitoring.

## Effectiveness of adalimumab in pediatric uveitis unrelated to JIA

A considerable proportion, approximately one-third, of the patients on conventional synthetic disease modifying antirheumatic drugs (csDMARDS) do not achieve sufficient disease control. In such "refractory" cases, the addition of biological agents like adalimumab, is recommended in the treatment of uveitis. The effectiveness of adalimumab for treatment of uveitis has been shown by multiple clinical trials, including the SYCAMORE trial, the ADJUVITE trial for adults with JIA-U, and the VISUAL I-III trial for non-infectious uveitis patients in adults. Adalimumab has received approval from the European Medicines Agency (EMA) for the treatment of pediatric patients with anterior uveitis. However, limited literature and research has evaluated the efficacy of adalimumab for the treatment of pediatric uveitis unrelated to JIA (non-JIA-U). In **Chapter 4**, we retrospectively investigated the effectiveness of adalimumab treatment in chronic pediatric uveitis unrelated to JIA. Similar to experiences in JIA-U, the addition of adalimumab to the treatment of pediatric patients led to significant decrease in disease activity and achieved disease inactivity in the majority (91%) of patients, resulting in improved visual acuity and a reduction of corticosteroid dosage. Adverse events and side effects were minimal.



There have been reports of demyelinating events in the central and peripheral nervous systems associated with TNF-α inhibitors, primarily in patients who already had white-matter abnormalities. The study in **Chapter 4** evaluated the use of adalimumab in a pediatric uveitis cohort and conducted brain MRI screenings prior to the start of adalimumab in 52% of the children without underlying systemic disease. These MRI analyses revealed a remarkably high percentage (up to 23%) of atypical white-matter abnormalities in the screened pediatric patients, which is significantly higher than the 2% usually reported in studies of unaffected controls. Of interest, cases that presented with white-matter abnormalities were either cases with intermediate or panuveitis.

## Biomarkers of vascular involvement

Nonanterior uveitis often involves complications of the retinal vasculature such as papillitis, cystoid macular edema (CME), retinal vasculitis, and retinal capillary leakage. Early identification of patients at risk of posterior involvement could facilitate prompt treatment to prevent vision-threatening complications. Currently, monitoring retinal vascular involvement relies completely on ad hoc clinical assessment, usually by imaging techniques, as there are no established molecular markers associated with these vascular complications. **Chapter 5** aimed to identify molecular signatures linked to retinal microcirculation involvement through targeted proteomic analysis of serum samples from children with noninfectious uveitis, utilizing a 384-plex targeted

proteomics panel (the Inflammation II panel from Olink). A total of 154 serum samples from noninfectious pediatric uveitis patients were compared to samples from 22 noninflammatory pediatric controls. The analysis revealed 63 differentially expressed proteins associated with platelet biology and the complement cascade. Notably, these differentially expressed proteins were primarily attributed to variations in serum proteomes between the anterior uveitis and nonanterior uveitis groups, the latter often characterized by retinal involvement. Further analysis in nonanterior cases identified 9 proteins specifically and significantly associated with retinal vascular involvement, particularly proteins enriched for the coagulation cascade. This suggests that mediators affecting microcirculation may play a role in noninfectious pediatric uveitis. Furthermore, our study provides evidence that coagulation and inflammatory processes may contribute to the pathophysiology of idiopathic uveitis in children.

## Involvement of the systemic microcirculation

In intermediate uveitis and panuveitis, retinal microcirculation can be affected, suggesting that these retinal abnormalities may be part of a broader systemic vascular pathology (see **Chapter 5**). This is supported by a study of Wennink and colleagues showing differently expressed serum proteins associated with systemic inflammation. In addition, in adults nailfold capillaroscopic abnormalities were observed in uveitis patients, which is a technique commonly used to investigate the peripheral microcirculation (see **Chapter 1** for details). In **Chapter 6,** we conducted an exploratory study to investigate the peripheral microcirculation in 119 noninfectious pediatric uveitis cases and 25 healthy pediatric controls using NFC. Significant differences in NFC parameters were observed between children with noninfectious uveitis and the pediatric control group; the pediatric uveitis cases exhibit a significantly higher number of dilated capillaries per millimeter and a higher prevalence of ramified capillaries compared to the control group. Additionally, the intermediate uveitis cases had a significantly higher number of dilated capillaries compared to the control group, whereas the anterior uveitis cases had a lower capillary density and a higher prevalence of ramified capillaries. Interestingly, no apparent correlation was found between abnormalities in the retinal microcirculation and in the nailfold microcirculation. Our findings suggest that the systemic microcirculation may be involved in non-infectious pediatric uveitis without an association with an underlying systemic disorder. Consequently, this study provides important new insights into the understanding of this severe and potentially vision-threatening ocular condition. Moreover, these findings serve as valuable input for designing future prospective and translational studies focused on microcirculation in pediatric uveitis.

# DISCUSSION AND FUTURE PERSPECTIVES

## Idiopathic CAU and JIA-U: a single disease entity?

Chronic anterior uveitis is currently divided into JIA-associated uveitis (JIA-U) and idiopathic chronic anterior uveitis (iCAU) as discussed in **Chapter 2**. In cases with iCAU, there is no arthritis or other systemic underlying condition, while there are many similarities regarding ophthalmological presentation, response to treatment, shared genetic factors, and proteomic profiles. These aspects will be further addressed below.

As mentioned above, idiopathic CAU cannot be distinguished clinically from JIA-U, based on ophthalmological characteristics.[1–3] However, iCAU patients do not undergo routine ophthalmologic screening for uveitis, leading to a higher occurrence of vision-threatening complications compared to JIA-U as is shown in this thesis (**Chapter 2**). Despite this difference, both groups exhibit similar long-term vision outcomes when appropriately treated with immunomodulatory therapy. Specifically, ANA-seropositive iCAU shares uveitis characteristics, clinical course, and response to systemic treatment with JIA-U, justifying the recommendation by recent therapy guidelines and the Childhood Arthritis and Rheumatology Research Alliance to teat JIA-U and iCAU equally.[4–7]



The presence of shared risk alleles (*HLA-DQB1*04:02 and HLA-DRB1*08:01*) in both iCAU and JIA-U further supports that iCAU is ophthalmologically identical to JIA-U.[8] While iCAU may in principle precede JIA-U, only a minority of patients with an initial onset of anterior uveitis develop arthritis later in life, likely due to the suppression of arthritis development through the successful treatment by immunomodulatory therapy.[8–10] A recent study of van Straalen and colleagues demonstrates a protective effect of methotrexate (MTX) on the development of new-onset chronic anterior uveitis in children with JIA.[11] This protective effect of MTX might also work the other way, i.e. for the development of arthritis in pediatric patients diagnosed with iCAU. It would be interesting to study the effectivity of MTX and other immunomodulatory therapy in children with presumed idiopathic CAU to prevent the development of arthritis.

Proteomic analysis provides another avenue for comparing isolated and JIA-associated CAU and for discovering potential biomarkers. A previous study from Haasnoot and colleagues has shown that iCAU and JIA-U cannot be differentiated by analyzing multicytokine profiles in aqueous humor,[12] which aligns with previous observations.[13–15] To overcome the invasiveness of collecting aqueous humor in children, Angeles-Han *et al.* investigated proteomics in tears,[16] and identified 29 differentially expressed proteins involved in extracellular exosomes in a small cohort of children with JIA-U

and iCAU. Examination of serum from pediatric uveitis patients suggests common immunoactivity, regulatory processes, and a shared adaptive disease mechanism for both disease entities.[15,17] Recent breakthroughs in proteomics are promising for identifying molecular signatures specific to uveitis subgroups, including JIA-U and iCAU. To further investigate this concept, we performed an additional analysis of the targeted proteomic analysis described in **Chapter 5** comparing 44 pediatric patients with JIA-U to 36 children with iCAU. We did not find any significantly different expressed proteins, suggesting that comparable circulating proteins are involved in the disease process despite the extraocular manifestations in the JIA patients.

In summary, iCAU demonstrates striking similarities with JIA-U in terms of clinical phenotype, treatment response, shared genetic background, and comparable proteomic profiles. Therefore, it is reasonable to consider iCAU as the same uveitis entity as JIA-U. To explore this even further the underlying molecular and cellular mechanisms of the two uveitis entities could be investigated.

## Optical coherence tomography angiography as a new application in pediatric uveitis

Fluorescein angiography (FA) is currently regarded as the gold standard for visualization of the retinal vasculature. Despite its relative safety, there are certain limitations and considerations associated with FA as described in **Chapter 1**. An alternative non-invasive technique for studying the retinal vasculature is OCT-angiography (OCTA). OCTA offers the advantage of segmented imaging of the flow of the choroidal vasculature in addition to the retinal vasculature, unlike FA which allows for clear visualization of superficial plexus vessel margins only.[18] This technique relies on high-frequency scanning to detect blood cell movement and has been used in children before.[19–27] However, studies using this tool in pediatric retinal vasculitis are lacking. Qu *et al.* conducted a retrospective study involving 32 pediatric uveitis patients and 30 matched healthy controls to investigate the utility of OCTA in pediatric uveitis.[28] The study revealed a reduction in vascular densities within both the superficial capillary plexus and deep capillary plexus of uveitis patients when compared to the control group. Notably, 29 of the 32 uveitis patients included in the study had idiopathic anterior uveitis, the other 3 cases were diagnosed with JIA-U. These findings highlight the need for further research utilizing OCTA to better characterize the specific features of pediatric retinal vasculitis. Such investigations will contribute to improved management strategies and prognostic capabilities, similar to adults.[29]

Preliminary OCTA data of our cohort of 42 pediatric patients with uveitis (80 affected eyes), showed lower values of vascular density, perfusion density, and peripapillary

perfusion density in pediatric uveitis patients compared to the healthy children of two other studies that used the same OCTA device (Cirrus, HD-OCT 5000; Carl Zeiss Meditec, Inc., Dublin, CA, USA).[19,30] Our hypothesis was that the retinal vascular abnormalities on OCTA would be associated with nailfold capillaroscopic abnormalities in children with pediatric uveitis. However, similarly to retinal vascular abnormalities observed on FA, we did not find an association when adjusting for age, sex and paired eyes. No differences were found with respect to uveitis subtype and uveitis activity. Our own preliminary study contains only a small sample size and does not include a healthy pediatric control group. Therefore, larger prospective studies are needed to investigate the diagnostic and prognostic value of OCTA in the management of children with pediatric uveitis.

The use of OCTA shows promising potential in pediatric uveitis, as it enables non-invasive visualization of the retinal vasculature and choriocapillaris. Consequently, OCTA was being considered as a replacement for conventional angiographic dye methods like FA. However, one significant limitation of OCTA is its inability to visualize the breakdown of the blood-retina barrier, a crucial biomarker in assessing retinal vasculitis. Therefore, as to date, OCTA cannot fully replace FA in daily clinical practice, but with further technological development it may reduce the need for conventual angiographic dye methods in the future.[31]



An important unmet need in uveitis management is the objective evaluation of intraocular inflammation. Existing clinical grading scales, such as those proposed by the SUN group[32], have limitations like low inter-operator agreement, poor repeatability, and the absence of a continuous scale.[18,33] Another limitation is the influence of the young child's cooperation on clinical grading. To address these issues, new techniques based on OCT analysis have been proposed. Anterior segment OCT has been used to count the number of cells and assess aqueous flare. Posterior segment OCT has been used to reliably measure vitreous haze. Additionally, new parameters like the choroidal vascularity index (which represents the ratio of vessel lumina to the total choroidal area)and vessel density measured by OCTAhave been suggested as supplementary tools for objectively evaluating the inflammatory status of uveitis.[18]

In the near future, there is a likelihood of a paradigm shift in the grading of intraocular inflammation, with objective measurements being performed in a fully, or at least partially, automated manner on digital images like OCT scans. This advancement will lead to better management in the everyday clinical practice and a higher accuracy in evaluating the efficacy of new drugs in clinical trials.[18,33]

## Biological agents in pediatric uveitis beyond JIA

Existing guidelines on initiating biological agents in pediatric uveitis are well-established; however, there is a lack of clear evidence and guidance on the appropriate discontinuation of these agents for patients with inactive disease.[6,34,35] Kalinina Ayuso *et al.* demonstrated that uveitis inactivity for at least 2 years before withdrawing MTX was related to a significantly longer relapse-free survival in patients with JIA-U.[36] This minimal duration of 2 years of inactive disease before withdrawal is often extrapolated to biological agents. The currently available literature on this topic primarily focuses on JIA. The heterogenicity of the study designs and the main outcomes of these studies hindered direct comparisons and definitive conclusions. Therefore, more robust research is needed to establish guidelines for discontinuing biological agents after achieving disease inactivity. A randomized controlled trail is presently underway to investigate the discontinuation of adalimumab in patients with controlled JIA-U.[37] Additionally, proteomics, which has been previously utilized to stratify clinical response to csDMARD therapy in pediatric uveitis,[38] may also prove valuable in identifying potential predictors and stratifying the risk of relapse after discontinuation of biological agents in pediatric uveitis. Furthermore, beyond disease and treatment characteristics, incorporating patient preferences through shared-decision-making is vital in devising an optimal treatment plan for each individual patient.

**Chapter 4** describes the efficacy of adalimumab in 43 pediatric patients diagnosed with non-JIA-U. To further explore the discontinuation of adalimumab in our own study, additional analysis showed that adalimumab was withdrawn in 10 of our patients (23%) due to disease inactivity of uveitis. Due to the limited number of patients, we can only provide a descriptive analysis of the results. Uveitis relapsed in 7 patients after a median time of 7 months after discontinuing adalimumab, in whom 4 (57%) adalimumab was restarted.

In our institute adalimumab in childhood uveitis is used in combination with a csDMARD.[34] Children frequently experience significant intolerance to MTX while on this combination. Therefore, it is not surprising that when discussing tapering of medication with patients and/or their parents, they often suggest to taper MTX instead of adalimumab, leaving a dilemma for choosing the subsequent therapeutic roadmap for physicians. A recent study shows the efficacy of adalimumab mono-therapy in non-infectious uveitis in children.[39] However, the long-term efficacy and the rate of adalimumab antibody development were not investigated in this study. Previous studies show that antibody formation occurred more in patients on adalimumab mono-therapy compared to patients with additional steroid and/or csDMARDs.[40,41] Moreover, the development of antibodies is related and frequently leading to treat-

ment failure.[41–44] The addition (or in most cases continuation) of a csDMARD is not only presumed to prevent antibody formation in uveitis patients but also contributes to disease controle.[40] Since the treatment options in patients with refractory pediatric uveitis are limited, adalimumab mono-therapy is not recommended by Dutch guidelines,[34] and should be considered in specific individual cases only.

Based on the current knowledge obtained in this thesis, we recommend prescreening with magnetic resonance imaging (MRI) of the brain before initiating adalimumab in children with nonanterior uveitis. However, the clinical relevance of aspecific white-matter MRI abnormalities is not elucidated yet. When performing brain MRI, there is a risk of incidental findings (IF). A meta-analysis of Dangouloff-Ros and colleagues shows a prevalence of 16.4% of IFs found on brain MRI within a large population of healthy children.[45] White matter hyperintensities, i.e. areas of abnormal myelination in the brain, occurred in 1.9% of the healthy children. The prevalence of white matter abnormalities in MRI of the brain in our study regarding nonanterior uveitis patients was almost 10 times as high. Note that the number of patients within our study was relatively small. Currently, it is unclear what these findings mean for the future of our pediatric population. Therefore, it would be important to further explore the prevalence, clinical characteristics and outcomes in a larger pediatric uveitis population and to gain more insight in the role of MRI screening.



## Uveitis: a local manifestation of a systemic disorder?

The exploratory study described in **Chapter 6** reveals nailfold capillaroscopic differences between pediatric uveitis cases and controls. This suggests involvement of the systemic microcirculation in children with uveitis. Previous studies have shown that several inflammatory markers such as the neutrophil/lymphocyte ratio, interleukins, and the platelet/lymphocyte ratio are elevated in the serum of patients with noninfectious uveitis. These increased markers are associated with both the activity and the severity of uveitis, likely implying general immune activation (i.e., an inflammatory index).[17,46–56] This thesis supports that during uveitis there are systemic changes in immune mediators detectable in extra-ocular tissues and the circulation. Currently, it is unknown what the abnormal NFC findings mean for the future of the pediatric population. Further investigation of repeated NFC measurements are needed in order to determine whether NFC findings have prognostic value with respect to predicting the course of disease severity in noninfectious uveitis. Furthermore, it would be interesting to investigate whether NFC findings can be used to predict disease relapse, the need for additional systemic treatment, and/or treatment response in patients with pediatric uveitis.

Another interesting factor that may influence the systemically inflammatory process is vitamin D. Vitamin D has many functions beyond its well-known role in calcium homeostasis and skeletal health, including a regulatory role in inflammation and infection.[57] Vitamin D modulates both innate and acquired immunity by limiting excessive inflammatory processes. It controls the expansion and differentiation of pro-inflammatory cells and reduces cytokine release.[57] Several studies have shown an association between low vitamin D levels and uveitis occurrence and severity.[58–61] However, these studies have not included pediatric uveitis cases, prompting our curiosity about the 25-hydroxyvitamin D status in this specific population. Preliminary data of our pediatric uveitis population, involving 63 non-infectious pediatric uveitis patients, showed that 64% of the patients had 25-hydroxyvitamin D levels ≤50 nmol/L, a common cutoff for vitamin D deficiency. No association was found between 25-hydroxyvitamin D levels and uveitis subtype, uveitis activity, or immunomodulatory therapy. To fully understand the role of insufficient 25-hydroxyvitamin D levels in uveitis, its potential for prevention and remission, and the effect of suppletion, large randomized controlled trials are required. This will help unravel the complex tolerogenic role of vitamin D in (pediatric) uveitis. First, a pilot study can be conducted to investigate a potential association between vitamin D and inflammatory molecular markers in pediatric uveitis patients without supplementation of vitamin D. Additionally, its relation to the complement system, a crucial component of innate immunity and involved in the pathogenesis of uveitis, can be investigated in (pediatric) uveitis. The complement system serves as a key mediator of immunity, defending against microorganisms and clearing apoptotic cells and immune complexes. Some common pathophysiological pathways have been observed in the musculoskeletal system, circulation, and metabolism, suggesting an interplay between vitamin D and the complement system.[62] However, this interaction remains unexplored in uveitis patients and warrants further investigation.

The genetic association of complement factor H (*CFH*) has been evaluated in relation to the development of uveitis. The *CFH*-rs1065489 polymorphism appears to be associated with anterior uveitis as well as uveitis recurrence.[63] There are many genetic variants or polymorphisms of the *CFH* gene in the general population, and therefore it would be interesting for future research to investigate whether these polymorphisms are also associated with retinal vascular involvement in idiopathic pediatric uveitis. Dysregulation of the complement system associated with genetic variants in chromosome 1 are associated with multiple ocular diseases such as age-related macular degeneration (AMD), central serous chorioretinopathy (CSCR), and multifocal choroiditis.[64–66] This suggests a common mechanism relating key factors of the complement cascade to retinal and choroidal abnormalities resulting in a differ-

ent presentation of ocular disease. Nailfold capillaroscopy has also been performed in AMD and CSCR showing alterations compared to healthy controls.[67,68] It would be very interesting to conduct a study performing NFC, proteomics, and genetic analysis of *CFH* polymorphisms, comparing ocular diseases with retinal vascular involvement, including AMD, CSCR and uveitis.

Targeted proteomics becomes more commonly available and allows for faster and accurate quantification in order to study biological pathways. Further studies regarding targeted proteomics in uveitis are warranted. Based on this thesis, future research is recommended involving Olink PEA proteomics or similar multiplex proteomics. To further investigate the role of coagulation and inflammation pathways in pediatric uveitis, other Olink PEA panels could be assessed and combined for different uveitis subtypes in order to find potential biomarkers that influence severity, disease course and response to treatment. Genetic variation between uveitis patients might also be associated with different biological pathways that involve coagulation, inflammation, and the complement cascade that could be studied through proteomics. Another interesting topic for future research would be to investigate prospectively the predictive value of the differentially expressed proteins of retinal vascular involvement. Several patients have increased levels of the differentially expressed proteins, but do not show clinical vascular retinal involvement established by imaging (OCT and FA) at time of sampling. These patients could develop retinal vascular involvement at a later stage, indicating a predictive value of the found proteins.



As a final suggestion, the proteomics data from **Chapter 5** could be combined with the Utrecht Patient Oriented Database (UPOD) data.[69] UPOD contains a combination of data for all patients treated at UMC Utrecht since January 2004, among which a specific database of hematology data obtained from a specific blood cell analyser.[69] This analyzer also determines data that are not standardly reported to the physician and could provide interesting information for research purposes. The exploration of the blood cell composition and morphological blood cell characteristics using UPOD data in combination with proteomics data could provide additional biomarkers regarding the microcirculation and vascular involvement in children with pediatric uveitis. Moreover, combining these data might create a prediction tool that could give more insight in the disease course of the individual patient and could lead to a more personalized treatment strategy that is more effective with less side-effects.

## Shared and multidisciplinary decision-making

A diagnosis of uveitis in childhood age should not be underestimated, as it can profoundly impact on the quality of life (QoL) of pediatric patients. These young patients

and their families often face significant physical, psychological and social challenges. The children may encounter disruptions in their schooling and academic progress, experience psychosocial and emotional stress, and have strained relationships with parents and siblings. Additionally, uveitis can affect their growth and development, cause severe fatigue, and lead to vision loss.[70–72] Currently, there is a lack of evidence on how to effectively address these issues in pediatric uveitis clinical services. Nevertheless, we want to underline the importance of considering the patients' QoL, along with its related challenges, and making it a key aspect of the management process. By giving due attention to these aspects, healthcare providers can better support the well-being of young patients and their families.

A multidisciplinary team consisting of an ophthalmologist, pediatrician/rheumatologist with expertise of immunological diseases is highly recommended and is standardized care in our tertiary hospital. This multidisciplinary approach not only applies for children with an underlying systemic disease, bot for all children diagnosed with pediatric uveitis. With the rapid development of immunomodulatory therapy and introduction of novel treatment options, an ophthalmologist often has insufficient knowledge on this topic. Additionally, the Dutch guidelines recommend that treatment with systemic immunomodulatory therapy of a pediatric uveitis patient should be in consultation with an expert on this field (immunologist, internist, rheumatologist) and the management of the disease should be in close collaboration.[34] This thesis provides evidence that the systemic microcirculation might be involved in pediatric uveitis. Therefore, a multidisciplinary approach of pediatric uveitis becomes even more important. At the end our aim is to provide the best possible care and maintain the best possible quality of life of the growing child and its family.

## CONCLUSIONS

In this doctoral thesis a vast breadth of factors that determine or improve the disease course of pediatric uveitis beyond JIA-U were studied with a main focus on microcirculation and vasculature. We provided an increase of knowledge about the clinical course of pediatric patients with chronic anterior uveitis. Furthermore, we proposed additional diagnostics and investigated novel treatment options in pediatric uveitis patients beyond JIA-U. We revealed an upregulation of circulating proteins with key functions in the coagulation and complement cascade in pediatric uveitis patients with retinal vascular involvement. Moreover, these data suggest that the microcirculation might play a role in noninfectious pediatric uveitis. Hence, we conducted exploratory research that revealed NFC abnormalities indicating involvement of the

systemic microcirculation in pediatric uveitis. This finding supports our believe that pediatric uveitis is not merely an isolated ocular inflammatory condition, but might be a manifestation of a disease affecting the systemic immune system and microcirculation. Moreover, it highlights the importance of a multidisciplinary approach in the management of pediatric uveitis patients to provide the best possible care.

This thesis emphasizes the importance of future research regarding the systemic involvement in all subtypes of pediatric uveitis with as main goal improving the prognosis and disease course. Research should especially focus on the pathophysiology of pediatric uveitis, which could lead to an increase of our understanding of this severe and inflammatory disease in children. Novel biomarkers associated with (retinal) vascular involvement are still of great importance, and might lead to innovative and more personalized diagnostic and therapeutic treatment options. Moreover, individual prediction tools could provide better insights in the disease course, preventing vision-threatening complications and improving the general prognosis for this vulnerable group of patients.

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# CHAPTER 8

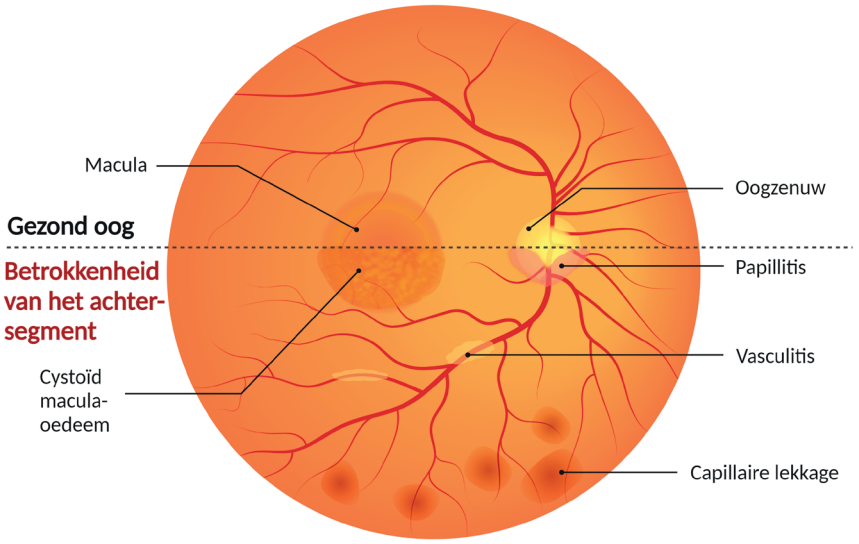
## Nederlandse samenvatting

Uveïtis is een inwendige oogontsteking van één of meerdere lagen van de *uvea (iris en vaatvlies (choridea)), een weefsellaag dat tussen de harde oogrok (sclera) en het netvlies (retina) ligt.* De retina en minder frequent de sclera kunnen ook bij het ontstekingsproces betrokken raken.

Uveïtis op kinderleeftijd is relatief zeldzaam en wordt gedefinieerd als het ontstaan van uveïtis voor het 16e levensjaar. Dit komt voor in 5-10% van alle uveïtisgevallen die worden behandeld in een gespecialiseerd (tertiair) verwijscentrum. Naar schatting lijdt 1 op de 1.000 kinderen in Nederland aan uveïtis. In de meeste westerse landen kan er geen infectieuze oorzaak worden gevonden voor uveïtis bij kinderen. In deze gevallen wordt gesproken over niet-infectieuze uveïtis. Wetenschappelijk onderzoek naar kinderuveïtis heeft zich tot nu toe voornamelijk gericht op niet-infectieuze uveïtis die geassocieerd is met jeugdreuma (juveniele idiopathische artritis-geassocieerde uveïtis, JIA-U). Hierdoor is er minder bekend over het klinisch management en de moleculaire verstoringen van andere vormen van uveïtis bij kinderen.

Lokale behandeling met corticosteroïde oogdruppels is meestal de eerste stap in het behandeltraject, maar heeft vaak onvoldoende effect en kan zelfs schadelijk zijn bij langdurig gebruik. Vanwege de ernst van de aandoening is het vaak noodzakelijk om intensieve systemische behandeling met immunosuppressiva, zoals methotrexaat, te starten om het ontstekingsproces tot rust te brengen en daaraan gerelateerde schade te beperken en het gezichtsvermogen te behouden. Bij een deel van de kinderen met uveïtis zijn de bloedvaten en microcirculatie in het oog bij het ziekteproces betrokken. Dit kan het ziektebeloop negatief beïnvloeden en de prognose verslechteren. Voorbeelden hiervan zijn onder andere (**Figuur 8.1**):

- **cystoïd macula-oedeem (CME):** lekkage van de capillairen en vochtophoping in de gele vlek of macula;
- **papillitis:** ontsteking van de oogzenuw, zichtbaar als een zwelling van de oogzenuw;
- **retinale vasculitis:** ontsteking van de bloedvaten in de retina;
- **lekkage van retinale capillairen:** lekkage van kleine bloedvaten (capillairen) in de retina door beschadiging en uitzetting van de bloedvaten zonder duidelijke tekenen van de inflammatie van de vaatwand zelf.




**Figuur 8.1** Vaatbetrokkenheid van het achtersegment bij kinderuveïtis. Afgebeeld staan cystoïd macula-oedeem, papillitis, retinale vasculitis en capillaire lekkage. Illustratie gecreëerd met Biorender.com.

De onderliggende moleculaire mechanismen van deze betrokkenheid van retinale bloedvaten bij kinderen met uveïtis zijn nog niet geheel duidelijk. Het is ook mogelijk dat niet alleen de oculaire bloedvaten bij het ziekteproces betrokken zijn, maar ook bloedvaten elders in het lichaam. De betrokkenheid van de systemische microcirculatie is nog niet onderzocht bij kinderen met uveïtis.

In dit proefschrift hebben we verschillende aspecten van kinderuveïtis zonder diagnose JIA bestudeerd. Het overkoepelende doel is om de huidige diagnostische en therapeutische strategieën voor kinderuveïtis te verbeteren. Deze nieuwe inzichten kunnen dienen als basis voor meer gepersonaliseerde interventies en kunnen bijdragen aan de ontwikkeling van nieuwe behandelingen. Dit hoofdstuk biedt een overzicht van de belangrijkste bevindingen van dit proefschrift in de Nederlandse taal.

## Klinische uitkomsten bij patiënten met idiopathische chronische anterieure uveïtis

Eerdere onderzoeken naar kinderuveïtis zijn voornamelijk gericht geweest op kinderen met JIA-U. Bij JIA-U komt vooral anterieure uveïtis voor. Dit heeft geleid tot een onvolledig beeld van anterieure uveïtis. Met name informatie over kinderen die gediagnostiseerd zijn met idiopathische chronische anterieure uveïtis (iCAU) ontbreekt. In **Hoofdstuk 2** beschrijven we een retrospectieve cohortstudie om dit kennishiaat

te verkleinen. We hebben de medische dossiers van 48 kinderen met iCAU en 48 kinderen met JIA-U onderzocht en gematcht op basis van leeftijd. We hebben hun klinisch beloop, visus, oogheelkundige complicaties en behandeling vergeleken. De voornaamste bevinding van deze studie toont aan dat kinderen met iCAU een complexer ziektebeloop hebben dan kinderen die gediagnostiseerd zijn met JIA-U. In het bijzonder hebben kinderen met iCAU een hogere prevalentie van synechiae posterior (verklevingen tussen de iris en lens), en een grotere kans op staaroperaties, zowel tijdens de diagnosefase als gedurende de follow-up periode. We vermoeden dat deze hogere complicatiekans ten opzichte van JIA-U verband houdt met een langere periode tot diagnose en adequate behandeling vanwege het vaak asymptomatische beloop van iCAU en ontbreken van screeningsmogelijkheden. Om deze hypothese nader te onderzoeken, voerden we een subgroepanalyse uit bij kinderen met JIA die eerst uveïtis en later artritis (gewrichtsontsteking) ontwikkelden. Deze analyse bevestigde dat de initiële metingen van JIA-patiënten die eerst uveïtis en later artritis kregen, vergelijkbaar waren met die van iCAU-patiënten.

Bovendien ontdekten we dat een jongere leeftijd bij diagnose (≤8 jaar) een risicofactor is voor oogheelkundige complicaties in het iCAU-cohort, wat overeenkomt met bevindingen bij JIA-U. Aan de andere kant hebben we geen verband kunnen vinden met het mannelijk geslacht als risicofactor, hoewel er in andere studies naar JIA-U ook geen sluitend bewijs is geleverd dat mannelijk geslacht een risicofactor is voor het optreden van oogheelkundige complicaties. Ondanks de verschillen in het optreden van visusbedreigende oogheelkundige complicaties tussen de twee groepen, bleef de visus op lange termijn goed, zonder significante verschillen tussen de groepen.



## Meting van de dikte van de retinale zenuwvezel laag op OCT voor diagnose papillitis

Fluoresceïneangiografie (FA) wordt beschouwd als de gouden standaard voor het detecteren van papillitis bij kinderen met uveïtis. Deze techniek is echter relatief invasief en vooral uitdagend in de uitvoering bij jonge kinderen. Het vaststellen van papillitis via fundoscopie is minder betrouwbaar vanwege verschillende complicerende factoren. In **Hoofdstuk 1** stelden we voor om de dikte van de retinale zenuwvezellaag gemeten met optische coherentietomografie (OCT-RZVL) te gebruiken als alternatieve niet-invasieve methode voor de beoordeling van papillitis. Om de diagnostische waarde van de OCT-RZVL-dikte voor papillitis bij kinderuveïtis te onderzoeken, voerden we een retrospectieve cohortstudie uit in **Hoofdstuk 3**.

Ten eerste voerden we een receiver operating characteristics (ROC) analyse uit om FA te vergelijken met OCT-RZVL in een subgroep van 93 kinderen met verschillende

subtypen van kinderuveïtis. Het optimale afkappunt voor de OCT-RZVL-dikte met zowel een relatief hoge sensitiviteit als specificiteit, werd vastgesteld op 130 µm. We voegden oogheelkundige gegevens toe, waaronder de aanwezigheid van cystoid macula oedeem (CME), ziekteactiviteit, en zwelling van de oogzenuw bij fundoscopie, als covariabelen aan het regressiemodel gebaseerd op de OCT-RZVL-waarden. Dit leidde tot een verbeterde capaciteit van het model om onderscheid te maken tussen ogen met papillitis en ogen zonder papillitis bij kinderen met uveïtis. Het lijkt daarom raadzaam om deze reeds beschikbare oogheelkundige gegevens op te nemen in de diagnostische aanpak voor een meer nauwkeurigere en niet-invasieve detectie van papillitis bij kinderen met uveïtis.

Vervolgens pasten we het vastgestelde afkappunt van 130 µm voor de OCT-RZVL-dikte toe op het volledige cohort van 257 patiënten met kinderuveïtis. Hieruit bleek dat iets meer dan een derde van de patiënten een OCT-RZVL-dikte had die groter was dan 130 µm. Zoals verwacht bestond de meerderheid van deze kinderen uit patiënten met een intermediaire of panuveïtis. Het meest voorkomende gezichtsvelddefect bij patiënten met een OCT-RZVL-dikte > 130 µm was een vergrote blinde vlek. De uitkomsten suggereren dat papillitis fungeert als een klinische indicator voor de activiteit van uveïtis. Daarom zou het vermogen om papillitis regelmatig te monitoren ons in staat kunnen stellen om de behandeling aan te passen, wat kan leiden tot een lager risico op blijvende visuele beperkingen. In conclusie is de meting van de OCT-RZVL-dikte een niet-invasieve, snelle en gemakkelijk uit te voeren methode bij kinderen, die waardevolle en toegevoegde inzichten kan bieden voor de ziektemonitoring.

## Effectiviteit van adalimumab in niet-JIA-geassocieerde uveïtis

Een aanzienlijk deel, ongeveer een derde, van de patiënten die conventionele synthetische disease modifying antirheumatic drugs (csDMARD) gebruiken, zoals methotrexaat, bereikt onvoldoende resultaat met deze behandeling. In zulke ‘refractaire’ gevallen wordt het toevoegen van biologicals, zoals adalimumab, aanbevolen voor de behandeling van uveïtis. De effectiviteit van adalimumab bij de behandeling van uveïtis is bevestigd in diverse klinische studies, waaronder de SYCAMORE-trial, de ADJUVITE-trial voor volwassenen met JIA-U, en de VISUAL I-III trials voor volwassen patiënten met niet-infectieuze uveïtis. Het Europees Geneesmiddelenbureau (EMA) heeft adalimumab goedgekeurd voor de behandeling van anterieure kinderuveïtis. Echter, onderzoek naar de werkzaamheid van adalimumab bij kinderen met niet-JIA-geassocieerde uveïtis is beperkt geweest. In **Hoofdstuk 4** hebben we een retrospectief onderzoek uitgevoerd naar de effectiviteit van adalimumab-behandeling bij kinderen met chronische uveïtis zonder JIA. In lijn met de bevindingen bij JIA-U, heeft het toevoegen van adalimumab aan de behandeling van kinderen met uveïtis geleid tot een

aanzienlijke vermindering van ziekteactiviteit, waarbij uveïtis in de meerderheid van de patiënten (91%) inactief werd. Behandeling met adalimumab leidde tot verbetering van de visus en vermindering van corticosteroïdengebruik. Er waren minimale bijwerkingen.

Er zijn gevallen gerapporteerd van demyelinisatie (afname of verlies van de myelineschede rondom zenuwvezels) in het centrale en perifere zenuwstelsel, die in verband zijn gebracht met tumor necrosis factor A-remmers, waaronder adalimumab. Demyelinisatie trad voornamelijk op bij patiënten die reeds bekend waren met witte-stof afwijkingen. Het onderzoek beschreven in **Hoofdstuk 4** heeft adalimumab-gebruik geëvalueerd in een cohort kinderen met uveïtis. Bij 52% van deze kinderen zonder geassocieerde systemische ziekte werd voorafgaand aan adalimumab-therapie een MRI-scan van de hersenen uitgevoerd. Deze MRI-beelden toonden opmerkelijk vaak (23%) atypische witte-stof afwijkingen bij de gescreende kinderen. Dit percentage ligt significant hoger dan het normaal beschreven percentage van 2% in studies met gezonde kinderen. Een interessante bevinding is dat de kinderen met witte-stof afwijkingen gediagnosticeerd waren met intermediaire of panuveïtis (niet-anterieure uveïtis).

## Biomarkers van vaatbetrokkenheid

Bij niet-anterieure uveïtis treden vaak complicaties op in de microcirculatie van de retina, zoals papillitis, CME, retinale vasculitis en lekkage van retinale capillairen. Het tijdig opsporen van patiënten die risico lopen op het ontwikkelen van deze vasculaire problemen van de retina kan ervoor zorgen dat de behandeling vroegtijdig kan worden gestart om visusbedreigende complicaties te voorkomen. Momenteel wordt het monitoren van retinale vasculaire betrokkenheid vaak gedaan op basis van ad hoc klinische beoordelingen, vaak ondersteund door beeldvormende technieken. Dit komt doordat er nog geen moleculaire markers zijn geïdentificeerd die verband houden met deze vasculaire complicaties. Het doel van **Hoofdstuk 5** is om een moleculair profiel te achterhalen dat verband houdt met betrokkenheid van de retinale microcirculatie. Voor dit onderzoek hebben we 384 eiwitten in het serum van kinderen geanalyseerd door gebruik te maken van ‘targeted’ eiwitanalyse van Olink (Inflammation II panel). We vergeleken serum samples van 154 kinderen met niet-infectieuze uveïtis met serum samples van 22 kinderen zonder inflammatoire aandoening.

De analyse onthulde verschillen in eiwitexpressie in 63 eiwitten die verband hielden met bloedplaatjes en het complement systeem. Deze verschillen kwamen voornamelijk tot stand door de variatie in serumeiwitten tussen de groepen met anterieure en niet-anterieure uveïtis. Verdere analyses, gericht op de samples van

niet-anterieure uveïtis patiënten, identificeerden 9 eiwitten die geassocieerd waren met retinale vasculaire betrokkenheid. Deze eiwitten waren vooral betrokken bij de bloedstollingscascade. Deze bevindingen suggereren dat mediatoren die betrokken zijn bij de microcirculatie mogelijk een rol spelen bij niet-infectieuze uveïtis bij kinderen. Daarnaast levert deze studie bewijs dat de bloedstolling en ontstekingsprocessen waarschijnlijk bijdragen aan de pathofysiologie van idiopathische uveïtis bij kinderen.

## Betrokkenheid van de systemische microcirculatie

Bij niet-anterieure uveïtis kan de microcirculatie in het netvlies aangedaan zijn. Dit doet vermoeden dat deze retinale afwijkingen mogelijk onderdeel zijn van een bredere vasculaire pathologie van het hele lichaam (zie **Hoofdstuk 5**). Deze hypothese wordt ondersteund door een onderzoek van Wennink en collega's, waaruit blijkt dat verschillende eiwitten in het bloed geassocieerd zijn met systemische inflammatie. Daarnaast zijn er nagelriemafwijkingen vastgesteld bij volwassen uveïtis-patiënten via nagelriem capillaroscopie (NFC), een techniek die vaak gebruik wordt om de perifere microcirculatie te onderzoeken (zie **Hoofdstuk 1** voor details). In **Hoofdstuk 6** hebben we een studie uitgevoerd naar de perifere microcirculatie bij 119 kinderen met niet-infectieuze uveïtis en 25 gezonde kinderen, gebruikmakend van NFC. We hebben significante verschillen in NFC-parameters waargenomen tussen kinderen met niet-infectieuze uveïtis en de gezonde controlegroep. De uveïtis-groep vertoonde een significant hoger aantal verwijde capillairen per mm. Dit verschil ten opzichte van de controlegroep was het grootste in de groep kinderen met intermediaire uveïtis. De uveïtis groep en specifiek de anterieure uveïtis had ook een hogere prevalentie van vertakte capillairen vergeleken met de controlegroep. Een andere specifieke bevinding voor de groep van de anterieure uveïtis is een lagere capillaire dichtheid (aantal capillairen per mm). Opvallend is dat er geen duidelijke correlatie is gevonden tussen afwijkingen in de retinale microcirculatie en de perifere microcirculatie. Deze bevindingen suggereren dat de systemische microcirculatie mogelijk betrokken is bij niet-infectieuze uveïtis. Daarnaast biedt deze studie waardevolle nieuwe inzichten in het begrip van deze ernstige en potentieel visusbedreigende oogziekte. Bovendien kunnen deze bevindingen dienen als belangrijke input voor toekomstige prospectieve en translationele studies gericht op de microcirculatie in kinderuveïtis.

## CONCLUSIES

In dit academische proefschrift is een veelvoud aan factoren onderzocht die het ziektebeloop bij kinderuveïtis zonder JIA bepalen of verbeteren, met speciale aandacht voor de microcirculatie en vasculatuur. Het biedt inzicht in het klinische beloop van kinderen met chronische anterieure uveïtis. Bovendien hebben we een voorstel gedaan voor aanvullende diagnostiek en onderzoeken we nieuwe behandelmogelijkheden voor kinderen met uveïtis zonder JIA. Onze bevindingen wijzen op verhoogde niveaus van circulerende eiwitten met betrokkenheid bij de stollings- en complementcascade bij kinderuveïtis-patiënten met retinale vaatbetrokkenheid. Deze gegevens suggereren tevens dat de microcirculatie een rol zou kunnen spelen bij niet-infectieuze kinderuveïtis. Om dit nader te onderzoeken, hebben we een onderzoek uitgevoerd die afwijkingen in de perifere microcirculatie aantoont, wat wijst op mogelijke systemische betrokkenheid bij kinderuveïtis. Deze bevindingen ondersteunen onze hypothese dat kinderuveïtis niet beperkt blijft tot een geïsoleerde inflammatie van het oog, maar eerder een uiting kan zijn van een aandoening die het systemische immuunsysteem en de microcirculatie beïnvloedt. Dit onderstreept het belang van een multidisciplinaire aanpak in de behandeling van patiënten met kinderuveïtis om op die manier de best mogelijke zorg te kunnen bieden.



Dit proefschrift benadrukt de noodzaak van toekomstig onderzoek naar de systemische betrokkenheid van alle subtypen van uveïtis, met als voornaamste doel het verbeteren van de prognose en het ziektebeloop. Speciale aandacht kan worden besteed aan het begrijpen van de pathofysiologie van kinderuveïtis, wat kan bijdragen aan een groter inzicht in deze ernstige inflammatoire aandoening bij kinderen. Nieuwe biomarkers geassocieerd met (retinale) vaatbetrokkenheid zijn van groot belang en kunnen leiden tot innovatieve en meer gepersonaliseerde diagnostiek en behandelopties. Bovendien zouden individuele predictiemodellen ons beter inzicht kunnen geven in het verloop van de ziekte, waardoor visus-bedreigende complicaties kunnen worden voorkomen en de prognose voor deze kwetsbare patiëntengroep kan worden verbeterd.

# APPENDICES

List of publications

Dankwoord (acknowledgements)

Curriculum vitae

# LIST OF PUBLICATIONS

## Related to this thesis

**Kouwenberg CV**, Wennink RAW, Shahabi M, Bozkir I, Koopman-Kalinina Ayuso V, de Boer JH. Clinical course and outcome in pediatric idiopathic chronic anterior uveitis. Am J Ophthalmol 2022;241:198-205. PMID: 35513031. doi: 10.1016/j.ajo.2022.04.015.

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# DANKWOORD (ACKNOWLEDGEMENTS)

Dit proefschrift was nooit tot stand gekomen zonder samenwerking, hulp en inzet van vele anderen. Ik wil daarvoor iedereen heel hartelijk bedanken. Zonder volledig te willen en kunnen zijn wil ik sommige mensen in het bijzonder noemen.

Allereerst wil ik alle patiënten en hun ouders bedanken die deel hebben genomen aan de klinische studies en/of op een andere wijze hebben bijgedragen aan de totstandkoming van dit proefschrift. Dit onderzoek is door hen en voor hen gedaan.

Daarnaast heb ik ontzettend veel geluk gehad met mijn promotieteam, die uiteraard onmisbaar is geweest: prof. dr. Joke de Boer, dr. Jonas Kuiper en dr. Viera Kalinina Ayuso.

Beste Joke, enorm bedankt voor de mogelijkheid dat ik dit proefschrift onder jouw hoede heb mogen schrijven. Ik vind het bewonderingswaardig hoe je ondanks je drukke agenda altijd tijd wist vrij te maken voor overleg. Ook was je altijd zeer snel met het kritisch beoordelen van mijn artikelen, wat erg heeft geholpen om de kwaliteit te verbeteren en de vaart er in te houden. Door je enorme kennis en laagdrempeligheid heb ik veel van je kunnen leren. Dankjewel voor je inzet om mijn promotie goed te laten verlopen, door bij te sturen, te bemoedigen en te inspireren waar dat nodig was.

Beste Jonas, jouw deur stond letterlijk altijd open voor een vraag of kort overleg. Bewonderingswaardig is je gave om de meest ingewikkelde procedures en technieken, wat voor gewone dokters vaak taaie kost is, eenvoudig uit te leggen en hiervoor ook altijd rustig de tijd te nemen. Jouw kritische blik en scherpzinnigheid maken dat het me is gelukt om dit mooie proefschrift af te leveren. Jouw (op moment van schrijven) pasgeboren kindje mag van geluk spreken met zo'n slimme en geduldige vader, ik wens jullie veel geluk en liefde samen.

Beste Viera, ik ben ontzettend dankbaar voor de kans die je me gegeven hebt om aan dit project te beginnen. Jouw passie, enthousiasme en gedrevenheid werken aanstekelijk en hebben bijgedragen aan een goede afronding van dit proefschrift. Ik heb een groot respect voor je kracht als persoon. Naast dat je me op wetenschappelijk gebied veel hebt geleerd, heb ik ook op persoonlijk vlak en in de spreekkamer veel van je geleerd. Je bent een ontzettend warme dokter, die echt hart heeft voor patiënten en hun naasten. Ik hoop in de toekomst nog veel van je te kunnen leren. Большое спасибо за все!

Beste commissieleden, beste prof. dr. Bleys, dr. Cheng, prof. dr. van Genderen, prof. dr. Jansonius, en prof. dr. Van Royen-Kerkhof. Bedankt voor uw energie en tijd om dit proefschrift te beoordelen en zitting te nemen in de commissie.

Ik wil natuurlijk alle coauteurs die hebben meegewerkt aan (een deel van) dit proefschrift bedanken, maar zou graag een aantal personen nog expliciet willen noemen.

Julia Spierings, ik ben ontzettend blij dat jij je bij ons team heb gevoegd. Je enthousiasme voor onderzoek, je kritische blik en je expertise op het gebied van nagelriemonderzoek en de reumatologie is van grote waarde geweest en heeft geleid tot de totstandkoming van hoofdstuk 6. Ik wil je ontzettend bedanken voor alle tijd en de vele uren die je hebt vrij weten te maken om mij te begeleiden bij ons gezamenlijke project en het beoordelen van de vele nagelriemfoto's. Ik heb veel respect hoe jij wetenschap, patiëntenzorg en een gezin weet te combineren. Femke van Rhijn-Brouwer, ondanks dat onze samenwerking maar van korte duur was, wil ik je graag bedanken voor je interesse en input voor dit proefschrift.

Mahfam, Laurens, Suzanne, en Mareike, door jullie als student te begeleiden heb ik zelf ook ontzettend veel geleerd. Bedankt voor jullie werk! Ik wens jullie heel veel succes met jullie carrières!

Graag zou ik Jan Boers willen bedanken voor de interesse in mijn promotieonderzoek en het ter beschikking stellen van de CapillaryScope, wat heeft geleid tot de totstandkoming van hoofdstuk 6.

Beste uveitiswerkgroep, bedankt voor jullie interesse en de mogelijkheid om de resultaten beschreven in dit proefschrift met jullie te delen.

Een groot woord van dank aan alle personen die ondersteunend zijn geweest in de uitvoering van het onderzoek in dit proefschrift. Suzan en Petra, hartelijk dank voor alle organisatorische hulp, onderwijs support, en antwoord op mijn vele vragen. Tamara, Marianne, Dax en Nivard, dank jullie wel voor het helpen bij het opzetten van de verschillende projecten, adviezen bij de regelgeving rond juridische- en METC-zaken, en de kritische blik bij het opstellen van de verschillende datamanagement plannen. Ferry van de Goede en Ruud Verweij, dank voor jullie hulp bij de ICT vraagstukken. Beste fotografen, hartelijk dank voor het uitvoeren van alle imaging en de moeite die jullie hebben genomen om mij dit alles uit te leggen.

Beste stafartsen en oogartsen in opleiding, heel veel dank voor de erg fijne samenwerking, jullie interesse in mijn promotieonderzoek, en jullie hulp als ik weer eens kwam vragen waar de iCare gebleven was. In het bijzonder wil ik de Elsbeth, Michelle en Rachel bedanken dat ik tijdens jullie OK's in de gelegenheid werd gesteld om bloed af te nemen bij de kinderen en meteen ook uitleg kreeg over de betreffende operatie. Beste orthoptisten (Aicha, Denise, Irem, Meike, Pascale en Sacha), hartelijk dank voor jullie interesse in mijn promotieonderzoek, jullie hulp bij de inclusies van de kinderen, jullie kennis en uitleg van het orthoptisch onderzoek en de gezellige praatjes. Jullie warme team is voor mij erg waardevol geweest. Ook Ina en Angelique wil ik bedanken voor jullie hulp en gezelligheid tijdens de kinderuveïtis spreekuren op de poli en voor het meten van alle flare's bij de kinderen.

In het bijzonder wil ik de lieve (oud) collega onderzoekers bij de oogheelkunde bedanken voor de goede sfeer, hulp en gezelligheid. Beste Kamil en Anna, onderzoekers van het eerste uur, dank voor jullie warme welkom. Mede door jullie voelde ik me meteen thuis. Sara, in het bijzonder wil ik jou bedanken voor je aanstekelijke enthousiasme voor wetenschappelijk onderzoek en voor al je goede tips en adviezen. Deniz en Els, helaas hebben wij maar kort samengewerkt, maar ik ben ervan overtuigd dat onze wegen elkaar nog zullen kruisen op oogheelkundige congressen. Roos, mijn voorganger op het gebied van kinderuveïtis, bedankt dat ik altijd bij je terecht kon voor vragen en voor de fijne samenwerking de afgelopen jaren. Aafke, Aroosha, Bas, Bram, Myrthe, Rianne, Sanne en Wouter, dank voor jullie hulp, de fijne werksfeer, gezelligheid en mooie momenten samen. Brendan, helaas lagen onze werkplekken wat verder uit elkaar, maar als AIOS hebben we genoeg tijd om elkaar beter te leren kennen. Evianne, wat hebben we samen een hoop tijd doorgebracht met het scoren van foto's. Zonder je ongekende positiviteit, kritische blik en adviezen had dit proefschrift er heel anders uitgezien. Lude (en Evianne), ik kijk terug op een ontzettend leuke tijd tijdens onze trip naar de ARVO in New Orleans, mijn beeld over crayfish zal nooit meer hetzelfde zijn. Janneau, onze gemeenschappelijke passie voor reizen maakt dat we vele uren hebben doorgebracht dromende over toekomstige reisplannen. Ik ben zeer benieuwd waar de volgende reis naartoe gaat! Cansu, bedankt voor je gezelligheid en interesse, en succes met het vervolg van je promotietraject. Casper, als kamergenoot wist jij me altijd te inspireren voor nieuwe sportieve doelen. Met die sporters mentaliteit ben ik ervan overtuigd dat jij je PhD tot een goed einde weet te brengen. Jytte, als medeonderzoeker op het gebied van kinderuveïtis kon ik altijd bij je terecht om even te relativeren. Ons tripje naar de IOIS in Berlijn was voor mij dan ook een mooi besluit van een boeiende en uitdagende reis die promoveren heet. Ik wens je nog veel succes met de afronding van je promotietraject!

Beste oud-collega's van de afdeling oogheelkunde van het Jeroen Bosch Ziekenhuis, graag wil ik deze kans aangrijpen om jullie te bedanken voor de mogelijkheid die jullie mij hebben geboden om kennis te maken met dit schitterende vakgebied. Zonder jullie enthousiasme en prettige samenwerking zou mijn carrièrepad er heel anders hebben uitgezien!

Een goede werk-privé balans is mijns inziens essentieel voor goed functioneren op de werkvloer. Ik wil graag alle vrienden en vriendinnen bedanken voor de leuke dingen die we gedaan hebben de afgelopen jaren. Het was een goede afleiding tijdens het onderzoek.

Lieve hechte helden, lieve Anne en Marel, Gerardo, Jaap en Naomi, Janneke en Remo, Karlijn en Ole, Luuk en Javi, Merijn en Eveline, Niels, Sifra, Teun en Simone, en Ward. Ik vind het heel speciaal dat wij al zo veel jaren, sinds de middelbare school, zo'n goede en hechte vriendschap hebben. Onze vriendengroep vakanties zijn inmiddels omgezet naar vriendenweekendjes en etentjes, maar nog altijd is onze vriendschap hecht. Of mensen nu in het buitenland wonen of slechts één straat verderop, wanneer we elkaar weer zien voelt het altijd zoals vanouds. Ik wil jullie allen ontzettend bedanken voor de interesse en afleiding de afgelopen periode.

Lieve Aaykie en Janneke, samen zijn we gestart aan onze studie biomedische wetenschappen. Nu zoveel jaar verder zijn we allemaal iets anders gaan studeren. Ik wil jullie bedanken voor jullie steun en betrokkenheid.

Lieve Annemarije, Eline, Thijs en Minette, oorsponkelijk zijn jullie vrienden van Stefan, maar inmiddels zijn jullie ook mijn vrienden geworden. Mede dankzij jullie steun, stimulatie en de nodige spelletjes avonden is dit proefschrift tot stand gekomen.

Lieve Levi en Loes, dank voor jullie oprechte interesse, lekkere high tea's en gezellige middagen. Helaas wonen we nu verspreid over het hele land, maar desondanks hoop ik dat we elkaar in de toekomst zullen blijven zien.

Lieve Maren en Leon, dankjewel voor jullie steun en voor de nodige afleiding. Onze gezamenlijke passie voor reizen maakt dat we vele uren samen hebben doorgebracht dagdromende over de volgende reis. Ik waardeer jullie vriendschap enorm en geniet van de vele gezellige avondjes en uitstapjes. Ik hoop dat we nog vele jaren samen lekkere ramen gaan eten, gezellige uitstapjes maken, naar kerstmarkten gaan en wie weet nog eens samen op vakantie gaan. Vielen Dank für Ihre Freundschaft!

Lieve Daniëlle, lieve paranimf, introgroepje 6, daar waar het allemaal begon. Inmiddels zijn we alweer bijna 15 jaar verder, maar ik ben nog altijd ontzettend dankbaar dat we elkaar tijdens de intro hebben leren kennen. Samen met Caroline, de cocktail lovers, al worden er de laatste tijd wel wat minder cocktails gedronken. Caroline, ik wil jou bedanken voor je steun en de gezellige uitjes. Daniëlle, ik ben erg blij en trots dat jij mijn paranimf bent. Ik wil je bedanken dat je altijd voor mij klaar staat, een luisterend oor biedt en dat we al vele leuke dingen samen hebben meegemaakt. Ik bof maar om jou, als ervaringsdeskundige, naast mijn zijde te hebben staan tijdens de promotie.

Lieve familie Heijmans en familie Kouwenberg, bedankt voor jullie intresse en betrokkenheid tijdens mijn promotieonderzoek.

Lieve schoonfamilie, lieve Nelly en Kees, Rens en Pauline, en Myrlon, ik wil jullie graag bedanken dat jullie mij hebben opgenomen in jullie familie. Jullie warmte maakt dat ik me bij jullie helemaal thuis voel en inmiddels zijn jullie mij heel dierbaar. De gezellige familieuitjes, Sinterklaas, Kerst- en Paasetentjes zijn altijd een hoogtepunt. Bedankt voor jullie oprechte interesse en betrokkenheid die jullie hebben getoond in mijn onderzoek.

Lieve Papa en Mama, ik prijs mezelf gelukkig met jullie als ouders. Ondanks dat het af en toe lastig was uit te leggen waar het onderzoek over ging of 'hoe dingen er aan toe gaan' bleven jullie geïnteresseerd. Jullie onvoorwaardelijke steun en vertrouwen geeft mij steeds de moed om dat stapje verder te zetten en is een bron van inspiratie en motivatie om door te blijven gaan. Ik realiseer me ten zeerste hoe bijzonder onze band is. Ik wil jullie dan ook in het bijzonder bedanken, voor jullie liefde, voor jullie steun, voor alles. Ik hou onwijs veel van jullie! Dit proefschrift is mede dankzij jullie zo geworden.

Stefan, allerliefste Stefan,

De afgelopen jaren hebben vele ups en downs gehad. Jij weet als geen ander dat dit nu eenmaal hoort bij het doen van onderzoek. Daarom besef ik maar al te goed dat onze beide promotietrajecten een grote rol in ons leven hebben gespeeld. Ondanks alles haal jij altijd het beste uit mij naar boven en bied jij mij onvoorwaardelijke steun en liefde. Ik denk dat er inmiddels geen oncoloog is die zoveel van (kinder)uveïtis afweet als jij. Ik wil je ontzettend bedanken dat je er altijd voor mij bent, voor je luisterend oor, en het relativeren. Jij bent de liefde van mijn leven, mijn maatje, mijn

alles. Ik hou onbeschrijfelijk veel van je en kan niet wachten om verder de wereld met jou te gaan ontdekken!

# CURRICULUM VITAE

Carlyn Kouwenberg was born on September 4th 1991 in Oss, the Netherlands. She is the only child of Gerry and Peter. Carlyn graduated from secondary school in 2009 at the Titus Brandsma Lyceum in Oss. In the same year she started studying Biomedical Sciences at the Radboud University in Nijmegen and obtained her bachelor degree in 2012. After a premaster year, she started her medical training at the same university. Throughout her years in Medical School Carlyn explored her interest in research at the department of neurology which resulted in several publications. In 2017, Carlyn obtained her medical degree and started working as a neurology resident (not in training) at the Jeroen Bosch Ziekenhuis in 's-Hertogenbosch. After a year of clinical experience, she decided to explore the field of ophthalmology by becoming an ophthalmology resident (not in training), first at the Bernhoven in Uden, and later at the Jeroen Bosch Ziekenhuis in 's-Hertogenbosch. This started her enthusiasm for ophthalmology.

In 2019 she started her PhD as described in this thesis under supervision of prof. dr. J.H. de Boer, dr. J.J.W. Kuiper and dr. V. Kalinina Ayuso. During her PhD, she presented her work on serval national (NOG, DOPS, Eilanddagen, Uveitis Werkgroep) and international conferences (ARVO, IOIS, IUSG), resulting in an award for the "Best Poster Presentation" at the Dutch Ophthalmology PhD Students (DOPS) conference in 2022. In addition to her research activities, she was secretary of the DOPS conference in 2021 and she supervised several medical students during their scientific internship.

Carlyn lives together with Stefan van Ravensteijn and their cat "De Majoor" in Berghem. She likes to spend her spare time hiking (in the nature reserve next-door), running (especially with friends), biking (on the Maasdijk), swimming (this passion started after finishing a quarter-triathlon together with one of her best fiends), or exploring the world with Stefan. Carlyn continued her journey in ophthalmology by starting her residency at the University Medical Center Utrecht in October 2023.