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# Clinical and immunological biomarker profiling to improve the diagnosis of acute uveitis

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# I. Abstract:

**Background:** Uveitis is a leading global cause of blindness. Common aetiologies include infectious or immune-mediated causes, but diagnosis can be delayed and remain challenging. Up to a third of patients have an undiagnosed aetiology and are classified as 'idiopathic'. Non-invasive biomarkers are urgently needed to expedite early accurate diagnosis in order to refine treatment and improve outcomes.

**Aims:** We sought to characterise clinical and immunological biomarkers which best discriminate between immune-mediated, infectious and idiopathic uveitis.

**Methods:** We prospectively recruited patients with acute uveitis from two tertiary centres in Sydney, Australia between June 2021 and February 2023. Clinical, visual, and serological data was collected in patient phenotyping. 35 cytokines, chemokines and growth factors were analysed in patients' serum samples and 30 age- and sex- matched healthy controls using a Milliplex® panel.

**Results:** 67 patients (37 female, median age 42 (range 16-79) years), were recruited with a median duration of follow-up of 18.5 months (range 12-37 months). 29, 17, and 21 patients were classified as immune-mediated, infectious, or idiopathic uveitis, respectively. Immune-mediated uveitis patients had a significantly shorter follow-up duration compared to infectious uveitis patients (24 months (4-37) versus 8.5 (4-33), p=0.028). A relapsing disease course was more common in the immune-mediated uveitis group (20/29, 69%), compared with the infectious group (3/17, 18%; p=0.004). 95 affected eyes (immune-mediated n=39, infectious n=24, and idiopathic n=32) were evaluated for visual acuity, retinal nerve fibre layer thickness, macular thickness, visual fields, macular oedema, and intraocular pressure at nadir and latest follow-up. Only macular thickness at nadir was higher in the immune-mediated group (median 270 μm, IQR 260-293) compared to the infectious group (250, 223-277, p=0.03).

Of the 35 cytokines/chemokines/growth factors tested, hierarchical cluster analysis and principle component analysis identified nine cytokines which best discriminated between uveitis patients and healthy controls; and six which best discriminated between immune-mediated, infectious and idiopathic uveitis. B cell-attracting chemokine 1 (BCA-1) was elevated in all uveitis groups compared to healthy controls. Patients with immune-mediated uveitis had higher interleukin (IL)-17α levels, while patients with infectious uveitis had lower IL-17α and growth-regulated oncogene-alpha (GROα) levels. Patients with idiopathic uveitis had elevated interleukin gamma-induced protein 10 (IP-10), tumour necrosis factor-alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1).

**Conclusions:** Cytokine/chemokine/growth factor analysis offers a non-invasive method for determining biomarker profiles in patients presenting with acute uveitis. We identified a refined panel of 9 biomarkers which drive differentiation between these groups and may be of diagnostic utility. These biomarkers may provide insights regarding underlying disease pathogenesis such as the role of B cell activation and migration to an immune-privileged uvea in all cases of acute uveitis, Th-17 as a major contributor to immune-mediated uveitis, impairment of neutrophil recruitment in infectious uveitis, and the role of Th-1 mediated inflammation in idiopathic uveitis. Idiopathic uveitis likely represents a heterogenous group of aetiologies, and further research regarding underlying disease mechanisms is required.

# II. Statement of originality

This is a statement to certify that the content of this thesis is my own work. This Thesis has not been submitted for any other degree or purpose.

I certify that the intellectual content of this thesis is the product of my own work, and that all assistance received in preparing this thesis and all sources have been acknowledged. My supervisors provided me with guidance and feedback to improve thesis writing skills. I have not published my work but I plan to publish it in the near future. Major part of cytokine analysis was undertaken by research co-supervisor Dr Magdalena Lerch.

Dr Serge Geara

Signed:

# III. Statement of Generative AI

This is a statement to state the software used and the extent of generative AI used in the thesis work below.

Generative AI was used via CHATGPT versions 3 and 4. The way it was used was to help me find resources for specific statements in the thesis, this was later checked manually by myself. Some questions regarding ways to use some of the statistics software and Microsoft Word were asked with CHATGPT. I made a query to CHATGPT regarding difficult topics in the thesis not covered by my prior knowledge or the literature clearly, specifically the section on anterior chamber autoimmune deviation, to help me understand and draft a clear section. I did not use generative AI to analyse any of the data directly, to ensure confidentiality of participants in the study and thesis that I did.

Dr Serge Geara

Signed:

# IV. Statement of government support

This research was supported by an Australian Government Research Training Program (RTP) Scholarship.

# V. Acknowledgments

I would like to thank my supervisors for their effort and patient to help me enter the challenging but exciting worlds of research, academic writing, and laboratory science. I would like to thank Professor McCluskey for working me through the theory in such a clear way, I am honored to be supervised by a world expert like you. Next, I would like to thank Professor Clare Fraser for her feedback and help and for setting the scene and the example for a do-it-all clinician researcher. I would like to thank Professor Elisa Cornish, who was very humble and always available to iron out any issue that might arise logistically or theoretically. Thank you for all the people who helped me collect samples, recruit, assess patients or just gave me access which include Dr Gabrielle Perry, Dr Gerard Reid and others. I would also have not been able to complete this project without the help of Dr Magdalena Lerch, who used her efficiency, precision, and expertise to help elevate this project to a higher level that it is now.

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I want to thank my parents for always being supportive and always thinking of me throughout this. I hope to continue the legacy of my father and siblings of being an academic person who does not settle for the current dogma, but continue to push the boundaries of knowledge.

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# Chapter 1: Introduction

## 1.1 What is uveitis

Uveitis refers to inflammation of the uveal layers of the eye, which encompass the iris, ciliary body, and the choroid (1). The uvea is the pigmented middle layer of the eye which is bound by the posterior surface of the cornea. Posteriorly the uvea's continuation is the choroid, which extends to the optic nerve head (2) (Figure 1). The eye has an anterior chamber, which encompasses the aqueous humour; and the posterior chamber, which includes the vitreous humour (3). The anterior chamber is separated into two segments with the demarcation being the iris. The anterior segment contains the iris and the ciliary body; the posterior segment extends from the back of the lens and includes the retina, choroid, optic nerve and posterior scleral coat. The iris and the ciliary body are involved in the production and circulation of the aqueous humour. The choroid provides nutrients to the posterior segment of the eye and retina (3, 4) (Figure 1.1). The pars plana acts as a physical barrier between the aqueous humour and the vitreous humour. It also helps maintain the intraocular pressure and shape of the globe. It also plays a minor role in aqueous humour production, through its non-pigmented epithelial cells(5). The posterior part of the choroid is considered the posterior uvea. These demarcations help us to understand and conceptualise uveitis (6).

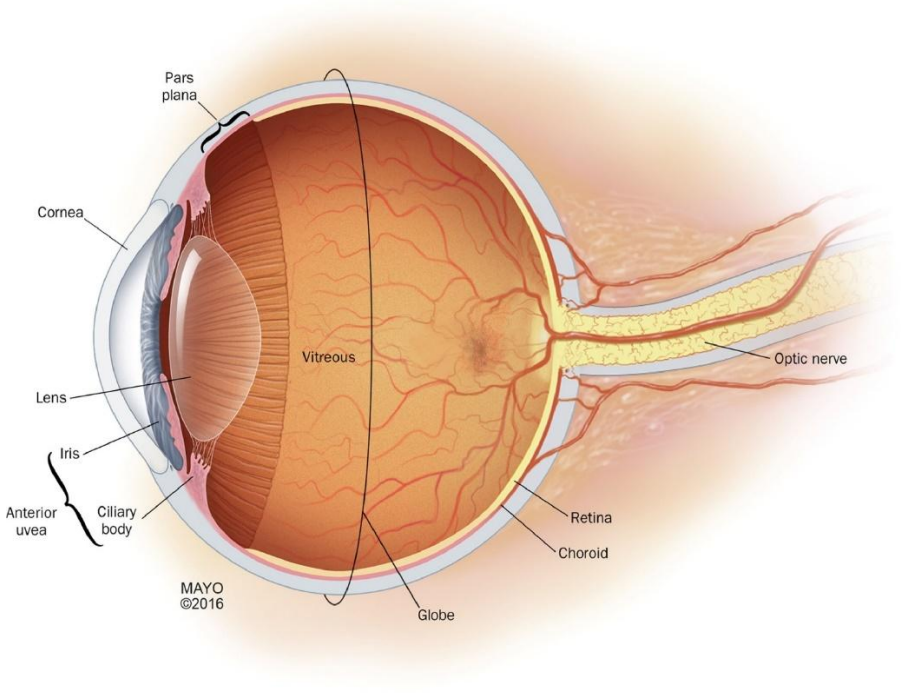



*Figure 1.1 Anatomy of the eye including the uvea, cross sectional figure (7).*

In some patients, uveitis may extend to involve inflammation of the optic nerve (resulting in optic neuritis) and the optic nerve sheath (optic perineuritis) (8).

## 1.2 Incidence and prevalence of uveitis

Uveitis has a reported incidence of 10-150/100000 patient years and a prevalence ranging between 54-300 person years (9-14). The wide range of these estimates is likely to be associated with the heterogeneity of aetiologies contributing to uveitis on a global scale, and the availability of diagnostic tools and specialised ophthalmological services in different centres (15, 16). Australian estimates are similar, mostly measured in tertiary centres. A study in 2019 in urban Melbourne in the acute tertiary care centre estimated the incidence at 21.54 per 100000 person-years and period prevalence of 36.27 per 100000 person years(17). Another critical factor which influences these

estimates is the increased prevalence of uveitis due to infectious aetiologies such as tuberculosis, in certain regions of the world such as South and Southeast Asia (18).

## 1.3 Global burden

Uveitis results in a significant burden from both a quality of life and health economics perspective. It is the fourth most common cause of blindness in working age adults from developed countries as defined by the World Health Organisation, and the fifth most common cause of blindness in Europe (19, 20). Most patients experience the onset of uveitis between the ages of 20-60, which is the most economically productive epoch of life. Similar cost analyses using comparable methodology, performed in the UK identified costs of 115 million euros annually arising from 40000 potential cases of blindness. A study of 12721 patients with uveitis across the 31 provinces of China found a correlation between higher incidence of uveitis and lower income in that province(21).

If left untreated, 10-20% of patients with uveitis can develop blindness impacting function (wherein a patient is unable to carry out many activities of daily living) as defined by a visual acuity of less than 20/200 or 6/60 in both eyes or a constriction of visual fields by less than 10 degrees (22-27). Furthermore, the ability to drive in Australia is more strict, with needing a visual acuity of 6/12 or better in both eyes, or the eye with better visual acuity(28). The tool used to measure visual acuity is a Snellen chart, and assesses a patient's ability to see a letter at 20 feet, (annotated as 20/20 vision) or 6 meters, (annotated as 6/6 vision) (29). The numbers used to define legal blindness correlate with functional outcomes (29).

The proportion of patients that develop blindness secondary to uveitis can rise up to 30% in developing countries as factors such as delayed recognition and treatment, and infectious

aetiologies, may adversely impact visual outcomes (18). A recent publication from Bogota, Colombia identified that in a cohort of 315 patients, it took an average of 2.08 years to be seen by a uveitis specialist (30). The diagnosis and treatment of uveitis may prove to be challenging as it requires an integration of history, clinical examination, and specialised investigations, and made harder by the fact that many symptoms in uveitis may be are nonspecific (31). Furthermore, the recognition and treatment of uveitis may be delayed by early presentations with subtle, or transient symptoms (32).

## 1.4 Classification of uveitis

Uveitis may be classified anatomically as well as due to its aetiology. The anatomical classification of uveitis has been proposed by the Standardised Uveitis Nomenclature (SUN) in 2005 (33) (Table 1.1). This was further refined in the SUN II update in 2021, and studied to include the most likely pathologies that can occur in the eye only (e.g. birdshot chorioretinitis) versus uveitis secondary to systemic diagnoses. Furthermore, the SUN group compared expert consensus with machine-learning algorithms in 5766 and found that in both classifications, there was 90%+ accuracy of diagnosis across all diagnoses.(34).

| location | primary site of inflammation | sites involved |
|---|---|---|
| anterior uveitis | anterior chamber | -iritis<br>-iridocyclitis<br>-anterior cyclitis |
| intermediate uveitis | vitreous | -pars planitis<br>-posterior cylitis<br>-hyalitis |

| | | |
|---|---|---|
| posterior uveitis | retina or choroid | -focal, multifocal, or diffuse choroiditis<br>-chorioretinitis<br>-retinochoroiditis<br>-retinitis<br>-neuroretinitis |
| panuveitis | a combination of above | |

*Table 1.1: Anatomical classification of uveitis. from Standardisation of Uveitis Nomenclature Standardisation of uveitis nomenclature working group classification taken from original classification in 2005. Standardisation of uveitis nomenclature for reporting clinical data (35).*

## 1.5 Anatomical classification of uveitis

### 1.5.1 Anterior uveitis

Anterior uveitis refers to inflammation of the anterior chamber of the eye (Figure 1.1). This includes the iris and ciliary body (14). It is the most common subtype of uveitis, encompassing about 50-80% of total cases of uveitis (9, 10, 12, 25). The presentation is usually that of redness (erythema), pain on eye movement, and sensitivity to light or photophobia, with the pain described as dull and aching (6). Visual acuity is usually not significantly affected in anterior uveitis, but patients can present with blurriness of vision (10). Anterior uveitis is less frequently sight threatening compared to posterior uveitis (36). While elevated intraocular pressure can occur in uveitis from various causes, it is a particularly suggestive feature of viral etiologies, such as herpetic uveitis (37).

Complications due to anterior uveitis may include cataracts, glaucoma and cystoid macular oedema (37). Cataracts may occur as a result of the uveitis itself, or a complication of corticosteroids used to treat uveitis. Uveitic glaucoma is initially managed with topical anti-glaucoma medications but can require surgery in advanced cases. Cystoid macular oedema is

potentially sight-threatening and an important complication to avoid. Its hallmark is the absence of the foveal reflexes. Treatments include topical nonsteroidal anti-inflammatory medications or intravitreal corticosteroid injections (38).

### 1.5.2 Intermediate uveitis

The largest studies of intermediate uveitis identify an incidence approximating 1.4/100000, and comprise 8 to 18% of all uveitis diagnoses, making it the rarest uveitis subgroup (5, 39-43). Intermediate uveitis refers to inflammation of the pars plana, anterior vitreous and occasionally the peripheral retina. Intermediate uveitis usually presents with floaters which are thought to represent the inflammatory cells in the vitreous, and associated blurriness of vision. Pain is often absent or delayed (5). The course of intermediate uveitis is commonly insidious at onset, with slow progression of visual signs and symptoms (44). Intermediate uveitis may be associated with concurrent anterior and/or posterior uveitis due to the close proximity of the pars plana with the choroid and the peri-retinal structures, including some extensions of retinal structures and/or cystoid macular oedema (45). Intermediate uveitis may have a “snowball and snowbank” appearance on fluoroscopy on fluorescein angiogram. The “snowbank” appearance is thought to reflect vitritis and “snowballs” are thought to reflect the inflammation of the pars plana (Figure 1.2) (46).

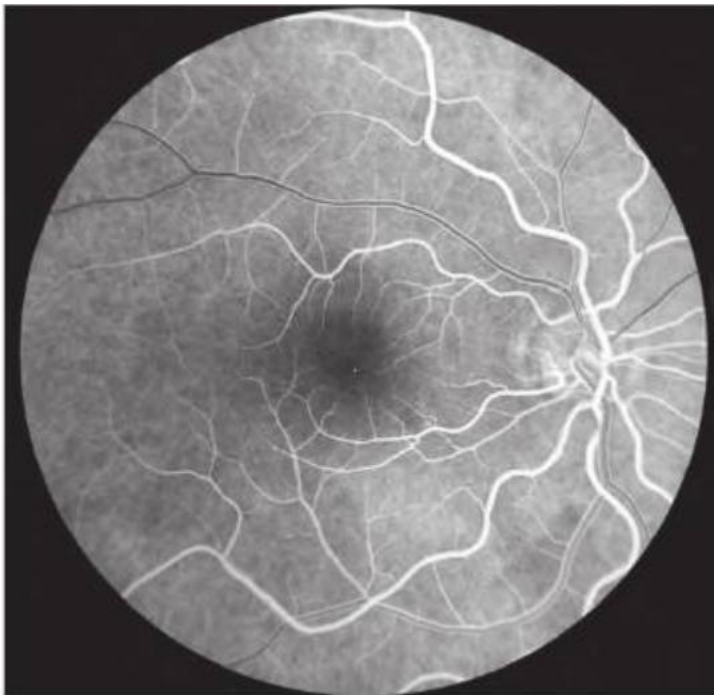


*Figure 1.2 Example of fluorescein angiogram in venous phase. Photo of a fluorescein angiogram showing normal venous structure(47).*

The hallmark of intermediate uveitis is vitreous inflammation and the presence of white cells seen on the slit lamp examination which indicates a breach of the blood retinal barrier and an active inflammatory process (5). The prognosis of intermediate uveitis depends on the underlying aetiology, but many patients retain their visual acuity at least at 6/12 (48).

### 1.5.3 Posterior uveitis

Posterior uveitis accounts for 10-15% of the total burden of uveitis (10). Posterior uveitis refers to inflammation of the posterior vitreous, the retina, or the posterior part of the choroid (49). Posterior uveitis generally needs to be significant or persistent to cause associated optic disc involvement (50). Concurrent optic disc involvement and associated optic neuritis, can cause significant drop in visual acuity, reduced colour differentiation (dyschromatopsia) and visual field loss. Its clinical manifestations are divided into retinitis, retinal vasculitis and choroiditis which includes diseases

that can involve the retinal pigment epithelium. The main hallmark of retinitis includes cellular infiltrates and necrosis of the retina which can be accompanied by oedema, and opacities of the vitreous cells (50). Retinal vasculitis may be associated with sheathing of blood vessels and occlusive retinopathy which manifests as loss of capillaries and cotton wool spots (51). This can lead to retinal haemorrhages and retinal ischemia (51).

Finally, choroiditis is divided into primary inflammatory choriocapillaropathies, primary stroma choroiditis and secondary stroma choroiditis (52). Primary choriocapillaropathies refer to disorders that arise from intrinsic dysfunction of the structures inside the choroid and capillaries. In contrast, secondary stroma choroiditis and inflammatory choriocapillaropathies refer to disorders that affect the tissue in question but arise from adjacent and systemic structures respectively. The techniques used to differentiate these types include fluorescein angiogram and indocyanine green angiography (ICGA) (53). Indocyanine green angiography (ICGA) is a different type of imaging technique that uses indocyanine green dye, which has a higher affinity for binding to plasma proteins and better penetration through the retinal pigment epithelium (RPE). This allows ICGA to visualize the choroidal vasculature, including deeper small capillaries, with higher resolution than fluorescein angiography (FA). It is particularly useful for diagnosing choroidal diseases(52, 54, 55).

In contrast, fluorescein angiography (FA) primarily assesses the retinal vasculature. Fluorescein dye can leak from abnormal blood vessels or pool in areas of fluid accumulation, helping to diagnose retinal pathologies (e.g., diabetic retinopathy or macular oedema). It can show different filling which depend on vascular flow dynamics:

-Arterial phase (~10–15 sec): Dye enters retinal arteries.

-Venous phase (~20–30 sec): Dye fills retinal veins.

-Late phase (>5 min): Dye may leak or pool in pathologic areas.

The timing, pattern, and shape of fluorescence (e.g., hyper-fluorescence from leakage or hypo-fluorescence from blockage) are key to determining the underlying disease (47). Examples of these pathologies are seen in figures 1.3, 1.4, 1.5 and 1.6.

Prognosis of posterior uveitis depends on the aetiology and the time of detection of the underlying pathology. The severity of cystoid macular oedema (CMO) in posterior uveitis is determined by chronicity and recurrence of inflammation, with prolonged or repeated episodes increasing the risk of irreversible vision loss. Additional factors include underlying uveitis etiology (e.g., Behçet's disease), delayed/inadequate treatment (leading to structural damage), and comorbid ocular complications (e.g., cataracts, glaucoma). Early, aggressive anti-inflammatory therapy (corticosteroids, immunomodulators, or local implants) is critical to mitigate severity.(49).

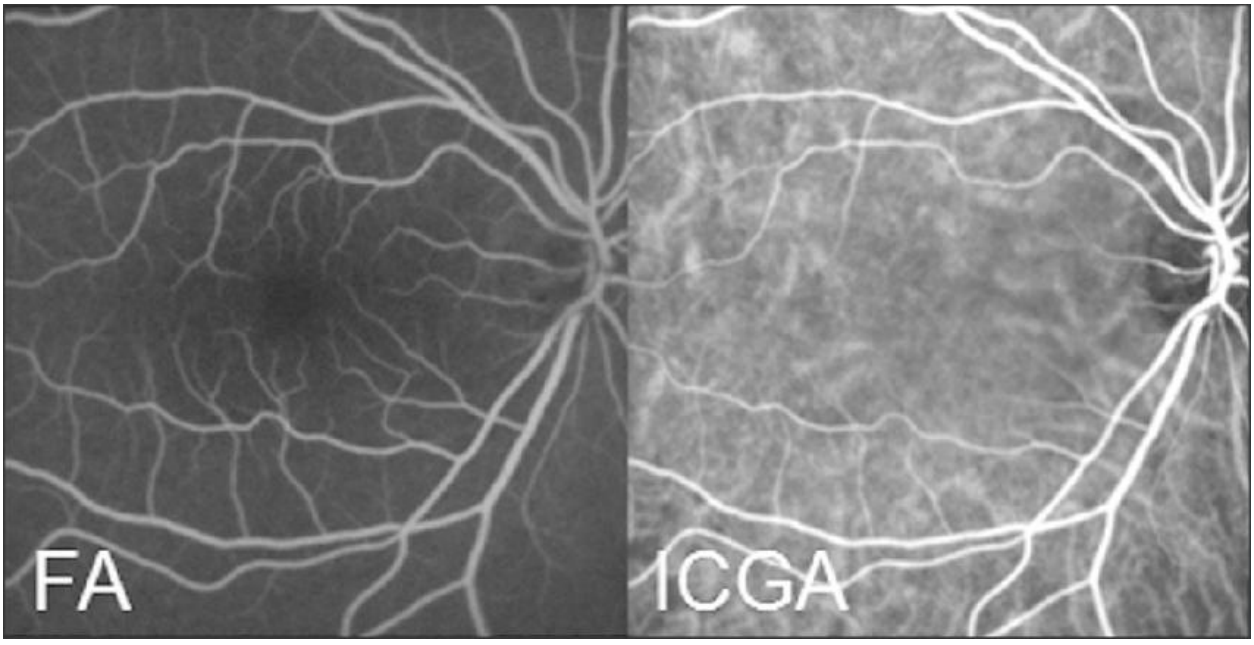


*Figure 1.3: Normal fluorescein angiogram and indocyanine staining of the right eye (56).*

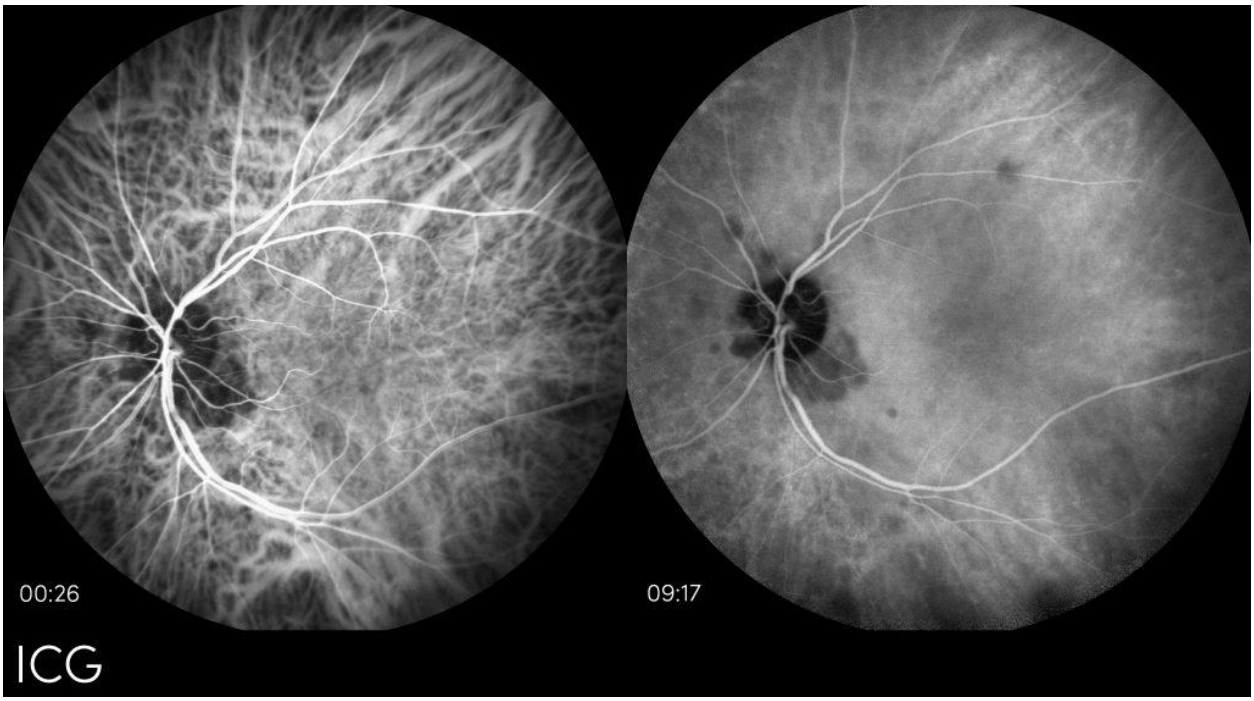



*Figure 1.4.Example of pathological Indocyanine staining. Indocyanine staining in a 45-year-old female with normal fundoscopy and decreased visual acuity showing hypocynesence suggesting a granulomatous aetiology as a differential. Patient was found to have tuberculosis with choroidal granulomas. (from(55)).*

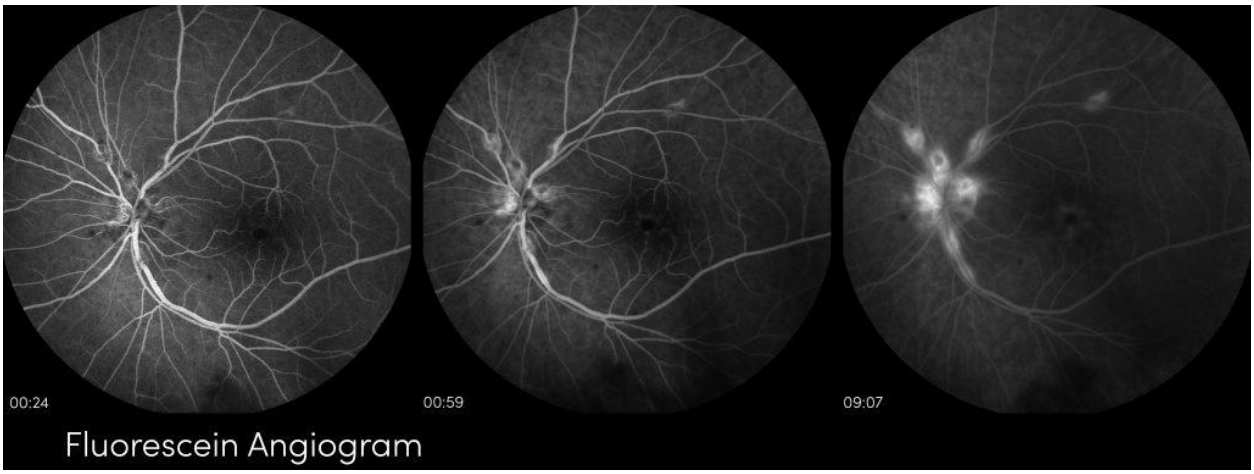



*Figure 1.5.Another example of pathology. Same patient as shown above. Three stages of fluorescein staining seen in this patient with perivascular staining indicating vasculitis (55).*

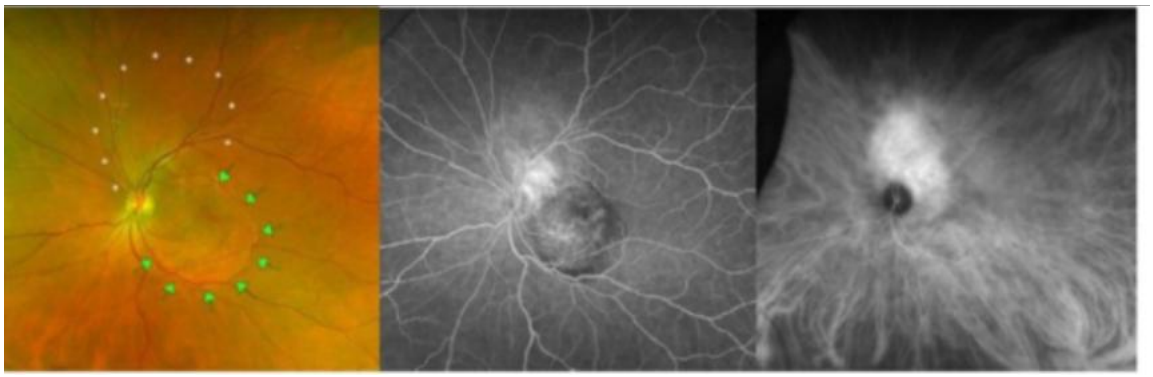


*Figure 1.6 A side-by-side comparison of the utility of indocyanine green angiogram. shows fundus photo (left), FA (middle), and ICGA (right). FA revealed hyperfluorescence from an ill-defined lesion superotemporal to the disc, while the ICGA showed diffuse and intense hypercyanescence of choroidal vessels, consistent with choroidal hemangioma (57).*

# 1.6 Aetiologies of uveitis

## 1.6.1 Infectious uveitis

Infectious uveitis refers to uveitis caused by bacteria, viruses, parasites or fungi, with more common causes being outlined below.

### *1.6.1.1 Syphilis*

Syphilitic uveitis is the main bacterial cause of anterior uveitis although syphilis may also present with optic disc swelling and posterior uveitis. It is caused by the bacterial gram-negative spirochete *Treponema pallidum* transmitted through sexual contact and mucosal surfaces (58). This infection results in ocular manifestations in all stages, but uveitis mostly occurs in the second stage of infection, called secondary syphilis. In addition to uveitis, syphilis may additionally manifest as keratitis, panuveitis, retinal vasculitis and chronic optic neuropathy (59).

Primary syphilis does not tend to cause uveitis as it does not involve the circulation of the pathogen (58). Untreated primary syphilis can lead to secondary syphilis in a period of 4-10 weeks which can have a multitude of presentations. The classic ocular presentation of syphilis is usually intermediate or posterior uveitis and is referred to as syphilitic necrotising retinitis (SRN). SRN is differentiated from other causes of retinal necrosis by its multifocal mottled appearance and its propensity to be seen more on the posterior pole on fluorescein angiogram testing, which also usually shows late vessel opacification. Finally, multiple snowball-like changes are seen in syphilitic posterior uveitis (60, 61).

Anterior syphilitic uveitis can be either granulomatous or non-granulomatous. Iris roseolae, ocular hypertension, placoid chorioretinopathy are all findings that can be found in primary and secondary syphilis that are highly specific and used for diagnosis (62). The treatment of syphilis includes 2-3 weeks of intravenous benzyl penicillin and topical or systemic corticosteroids. Other considerations for duration of treatment include concurrent immunosuppression and the severity of systemic manifestations (63).

### *1.6.1.2 Tubercular uveitis*

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis,* an acid-fast obligate aerobe bacterium (64). TB mainly affects the lung parenchyma, however it has multiple organ manifestations, including the uvea (65). TB is the most common cause of uveitis in some endemic areas such as India, accounting for up to 20% of the total uveitis burden (66). TB can cause granulomatous anterior uveitis with mutton-fat keratitic precipitates, and synechiae (67). Anterior TB uveitis accounts for 36% of the total TB uveitis (68). The diagnosis is more accurately made

in the anterior chamber with a tissue diagnosis showing positive acid-fast bacilli or caseating granuloma, which differentiate it from other causes such as sarcoidosis (69).

In the posterior chamber, there are more diverse manifestations of tissue predilection and morphology. Posterior chamber uveitis accounts for 40% of tubercular uveitis (68). Multifocal choroiditis or serpiginous-like choroiditis is suggestive of tubercular involvement. Furthermore, tuberculomas and subretinal abscesses can develop in the posterior uvea and may be associated with meningitis (70). Treatment includes steroids if vasculitis or choroiditis develops, and standard quadruple therapy with rifampicin, isoniazid, pyrazinamide and ethambutol (71).

#### *1.6.1.3 Toxoplasmosis*

Toxoplasmosis uveitis is a form of ocular inflammation caused by the parasite *Toxoplasma gondii*. It is one of the most common infectious causes of posterior uveitis worldwide. Toxoplasmosis uveitis typically presents with unilateral ocular symptoms, although bilateral involvement can occur. The classic clinical features include focal retinochoroiditis, often located adjacent to the optic nerve or in the macula. The active retinochoroiditis lesion appears as a whitish-yellow necrotic area with overlying vitritis, resulting in blurred vision, floaters, and decreased visual acuity. Recurrent episodes of inflammation may lead to the formation of chorioretinal scars, resulting in permanent vision loss if located centrally. The diagnosis of toxoplasmosis uveitis relies on a combination of clinical examination findings, laboratory tests, and imaging studies. Ophthalmic examination reveals characteristic features such as focal retinochoroiditis with vitritis and chorioretinal scars (72-74). Ancillary tests, including serologic testing for toxoplasma-specific antibodies (immunoglobulin (Ig)G and IgM), polymerase chain reaction (PCR) assays, and intraocular fluid analysis (aqueous or vitreous tap), can aid in confirming the diagnosis and

detecting active infection (72-74). PCR assays performed on intraocular fluid samples allow for the direct detection of toxoplasma DNA, providing definitive evidence of active ocular infection. This molecular diagnostic technique is particularly useful in cases where serologic testing results are inconclusive or in patients with atypical clinical presentations, particularly in the immunosuppressed patient (74). Treatment usually includes antiparasitic medications and oral steroids (75).

#### *1.6.1.4 Viral uveitis*

##### *a Herpes simplex virus (HSV)*

HSV 1 and 2 belong to the *Herpesviridae* group of viruses and are a major cause of uveitis. The most common manifestations include keratitis and anterior uveitis, and may be accompanied by either granulomatous or non-granulomatous pathology (76). Common findings in HSV anterior uveitis include dilated iris blood vessels with a flat pupil. Another typical change is keratitic precipitates which usually correspond to moderate anterior chamber activity (77). Iris atrophy is correlated with aqueous humour viral load , which can be measured with a vitreous aspiration (76). Finally, all viral, including HSV uveitis, can cause hypertensive uveitis(78). Acute retinal necrosis is a recognised complication of untreated HSV, more evident with HSV-1 than HSV-2 (79),this can also be seen in VZV as well. A typical presentation of all viral posterior uveitis is that of panuveitis which starts as vitritis and vasculitis. It spreads from the “outside in” within a few days up to 3 weeks and typically causes vitritis and retinitis. Prognosis depends on how early it is detected after symptom detection, whether or not it involves the retina, the length of treatment, as well as the level of immunosuppression. If it becomes bilateral and not treated promptly, it could lead to significant complications including blindness (80).

| | HSV uveitis | VZV uveitis | CMV uveitis | chronic CMV uveitis |
|---|---|---|---|---|
| **age** | 30–50 years | 50–70 years | 20–50 years | 40–70 years |
| **sex** | equal | equal | males (65%) | males (80%) |
| **race** | all | all | predominantly Asian | predominantly Asian |
| **laterality** | bilateral in 18% of eyes | unilateral | unilateral | predominantly unilateral |
| **skin involvement** | ± crops of vesicles | ± dermatome blisters | none | none |
| **cornea** | scars 12%–33% corneal involvement 57%–61% | scars 2.5%–9% corneal involvement 58% | ± nodular endothelial lesions 26% | ± nodular endothelial lesions 60%, immune ring |
| **corneal sensation** | reduced | reduced | normal | normal |
| **KP** | granulomatous or non-granulomatous | granulomatous or non-granulomatous | granulomatous | fine, stellate, diffuse ± pigmented |
| **AC cells** | moderate | moderate | few | moderate |
| **AC flare** | moderate | moderate | minimal | minimal |
| **pupil shape** | may be irregular | may be irregular | normal | normal |
| **posterior synechiae** | may be present 25%–38% | may be present 0%–40% | absent | absent |
| **iris atrophy** | sector or spiral 25%–46% | sector, circular 25%–88% | patchy or diffuse, rarely sector 43% | diffuse or patchy 60% |
| **IOP** | elevated 38%–90% | elevated 40%–75% | elevated 100% | elevated 69% |
| **vitritis** | 43% | 83% | 0% | 9% |
| **glaucoma** | present in 18%–54% | present in 30%–40% | 23% | 36% |
| **cataract** | present in 28%–35% | present in 27%–30% | 23% | 75% |
| **recurrence** | in 15%–65% | in 13%–51% | 100% | 0% |

Table 1.2: *Comparison of the epidemiology, clinical features, complications, and clinical course of uveitis among the three herpes viruses (76). Abbreviations: AC: anterior chamber, CMV: cytomegalovirus, IOP: intraocular pressure, HSV: herpes simplex virus; KP: kertatic precipitate, cranial nerve V1: trigeminal nerve first section, VZV: varicella-zoster virus.*

*b Varicella zoster virus (VZV) uveitis*

VZV uveitis is a rare but significant cause of intraocular inflammation, typically occurring in individuals with a history of varicella (chickenpox) or herpes zoster (shingles). It presents as anterior uveitis, posterior uveitis, or panuveitis and may involve multiple ocular structures, leading to vision loss if left untreated. Diagnostic modalities include clinical examination findings, PCR assays of intraocular fluid samples, and serologic testing for VZV antibodies (81). Treatment involves antiviral medications, corticosteroids, and in some cases, immunosuppressive agents, depending on the severity and extent of inflammation (82).

*c Cytomegalovirus (CMV) uveitis*

Cytomegalovirus (CMV) uveitis is secondary to an infection with the herpesvirus CMV. CMV uveitis is more common in immunocompromised patients, and patients who have comorbidities such as a renal transplant, dialysis, uncontrolled diabetes or human immunodeficiency virus (HIV) infection (83). It may present associated with keratitis and retinitis (83). Diagnosis is via an intravitreal tap with PCR (84). A specific severe form of CMV associated anterior uveitis is called Posner-Schlossman syndrome which can cause rapid loss of vision(85). In contrast to VZV and HSV, CMV uveitis is associated with coin shaped keratitic precipitates and a decreased number of endothelial cells in most patients (76). The prognosis of CMV relies on the comorbidities and level of immunosuppression but often carries a worse prognosis than HSV and CMV uveitis (76).

*d Human Immunodeficiency virus uveitis*

HIV associated uveitis represents a spectrum of ocular inflammatory manifestations seen in individuals living with HIV/Acquired Immunodeficiency Syndrome (AIDS). It typically presents

as bilateral, anterior uveitis with granulomatous features, although posterior segment involvement can occur. It is believed to involve dysregulation of the immune response secondary to HIV infection. $CD4^+$ T-cell depletion and immune reconstitution inflammatory syndrome (IRIS) are contributing factors (86-88). Posterior intracorneal opacities can sometimes aid diagnosis and usually it is a manifestation of severe immunocompromise. Prompt recognition and treatment are crucial to prevent irreversible visual impairment (86-88).

## 1.6.2 Immune-mediated uveitis

Immune-mediated uveitis encompasses a spectrum of inflammatory eye diseases characterised by intraocular inflammation driven by aberrant immune responses. Uveitis is not an uncommon manifestation of neuroimmunological disorders, with 7.9% of patients with neurological disorders having uveitis as an associated manifestation (23).

### *1.6.2.1 Vogt-Koyanagi-Harada (VKH) disease*

VKH disease is a bilateral, granulomatous uveitis associated with systemic manifestations. Findings include bilateral panuveitis, serous retinal detachments, optic disc hyperaemia, and sunset glow fundus, with the potential for vision-threatening complications. Management involves systemic corticosteroids to suppress inflammation and immunomodulatory agents for long-term control. Early recognition and aggressive treatment are crucial to prevent vision loss and systemic complications (89).

### *1.6.2.2 Human leukocyte antigen (HLA)-B27 related uveitis*

*HLA-B27 i*s a major histocompatibility complex class I gene, and its association with various autoimmune conditions, including uveitis, has been well-established (90, 91). The prevalence of

HLA-B27 varies among different populations, and the gene is found more frequently in individuals of European descent and some specific Han Chinese populations (90, 92). The genetic link suggests a role for the immune system in the development of uveitis, and HLA-B27 testing is often conducted in patients with uveitis to aid in diagnosis and guide treatment strategies (93-95).

HLA-B27-associated uveitis typically presents with acute, recurrent, or chronic inflammation. The condition can affect one or both eyes and may manifest with various ocular symptoms, including redness, pain, photophobia (sensitivity to light), and blurred vision (96). Most anterior uveitis in European and Caucasian population is felt to be HLA-B27 related (91, 92). One distinctive feature of HLA-B27-associated uveitis is its association with certain systemic conditions, particularly seronegative spondyloarthropathies (95). Conditions such as ankylosing spondylitis, reactive arthritis, and psoriatic arthritis are more prevalent in individuals with HLA-B27 and may coexist with uveitis (95). Recent research has revealed correlations between risk of uveitis with and the gut microbiome and certain infections such as *Chlamydia trichomatis* being associated with higher incidence of activation of HLA-B27 related disease, which has implications on treatment (95). While HLA-B27-associated uveitis tends to have a recurrent and episodic course, the overall visual prognosis is generally favourable with appropriate management despite the incidence of complications being around 40% (96).

*1.6.2.3 Juvenile idiopathic arthritis (JIA) associated uveitis*

Uveitis is an extra-articular manifestation that can be associated with JIA (97). Typically, it presents as chronic anterior uveitis, often bilateral and insidious, with symptoms including eye redness, pain, and decreased visual acuity, although most commonly it is asymptomatic(98). If left

untreated, it can lead to complications like synechiae, cataracts, glaucoma and vision loss (99, 100). The prognosis varies, with some patients experiencing persistent inflammation despite treatment, while others achieve remission. Notably, early detection and aggressive systemic management are crucial for preserving vision (97).

*1.6.2.4 Behcet's disease associated uveitis*

Behcet's disease is a systemic immune syndrome with a classic manifestation of recurrent posterior uveitis, oligoarthritis, genital and oral ulceration and dermatitis; as well as neurological manifestations in some patients. It has an increased prevalence in certain ethnicities, mostly around the Silk Road from China to the Mediterranean Basin. It is associated with the HLA-B*51 haplotype (101). It has a higher predominance in Middle Eastern and near East Asian populations (102). Its prevalence is determined by the population with up to 420/100000 in Turkish populations (103). Uveitis occurs in 50% of patients and is associated with worse outcomes. It is bilateral in 80% of patients (104). Superficial retinal infiltrates with occlusive retinal vasculitis and capillary leakage, without any granulomatous anterior uveitis in patients with vitritis is highly suggestive of the condition (105). It is treated with immunosuppressive agents that can range from corticosteroids to systemic immunosuppression and tumour necrosis factor (TNF)-α inhibitors. For severe manifestations, early treatment and the use of monoclonal antibodies confer the best prognosis (104).

*1.6.2.5 Multiple sclerosis*

Multiple sclerosis (MS) is a relapsing immune disorder characterised by demyelination in the central nervous system. MS can have multiple manifestations including the presence of plaques in the brain, spinal cord, and optic nerve (106). Classic syndromes related to MS did not include

uveitis, but it now recognised that patients with MS can be five times as likely to get uveitis compared to the general population, making a link more likely (40). Retrospective studies estimate that about 1% of all MS patients can have uveitis, and 1% of all patients who present with uveitis have an ultimate diagnosis of MS (107). MS-related uveitis is commonly an intermediate uveitis (around 80%) with anterior uveitis being second most common at 15% sometimes accompanied by associated retinitis (40, 108).

*1.6.2.6 Myelin oligodendrocyte glycoprotein (MOG) antibody-associated uveitis*

MOG antibody-associated disease is a demyelinating disorder characterised by positive MOG antibodies in the serum, and a typical clinical profile associated with optic neuritis, transverse myelitis, acute disseminated encephalomyelitis (ADEM) and other less frequent manifestations including cerebral cortical encephalitis (109).

Uveitis associated with MOG antibodies is a relatively newly recognised entity, characterised by uveitis often accompanied by optic neuritis (110). This uveitis can be anterior, posterior or intermediate. Studies have highlighted the importance of recognising MOG antibody-associated uveitis as a distinct clinical entity, with a favourable response to corticosteroid therapy (110-112). The cases are limited in literature, but patterns emerge. MOG antibody related uveitis has also been identified following syphilis and CMV infection (113, 114).

*1.6.2.7 Multiple evanescent white dot syndrome (MEWDS)*

MEWDS is a rare and distinctive ophthalmic condition that primarily affects the retina. Described by the presence of multiple small, white dots in the posterior pole of the eye, MEWDS is

commonly found in young female adults. This syndrome manifests with acute visual disturbances, including visual field defects and photopsias. While the precise aetiology of MEWDS remains incompletely understood, it is hypothesised to involve an inflammatory response impacting the outer retina. The condition typically follows a self-limiting course, and most individuals witness spontaneous resolution of symptoms within weeks to months (115).

#### *1.6.2.8 Birdshot chorioretinitis*

Birdshot chorioretinopathy (BSCR) is a rare and chronic inflammatory eye disease that primarily affects the choroid and retina (116). This condition typically presents with distinctive, hypopigmented choroidal lesions resembling birdshot pellets, leading to visual impairment and, if left untreated, potentially causing permanent damage. BSCR primarily affects individuals of European descent (117) and is often associated with HLA-A29 positivity (118). The exact etiology of BSCR remains unclear, but it is considered an autoimmune disorder. Diagnosis involves characteristic clinical findings, including the appearance of birdshot lesions on fluorescein angiography and indocyanine green angiography. Treatment options include corticosteroids and immunosuppressive agents, with varying success rates (116).

#### *1.6.2.9 Sarcoid uveitis*

Sarcoidosis is a systemic granulomatous immune disease (119). Its most recognised feature is bilateral hilar lymphadenopathies, however it has neurological manifestations such as central white matter disease, myelopathy, neuropathy and can be associated with ophthalmological manifestations (120).

Sarcoid uveitis affects all regions of the uvea and can be bilateral (121). Clinical findings include slow subclinical vasculitis and muttonfat-keratitic precipitates, which suggests a granulomatous process (122). Treatment includes TNF-α inhibitors, topical and systemic corticosteroids (123).

### 1.6.3 Environmental posttraumatic uveitis

Patients can be exposed to medications that can be associated with uveitis (Table 1.3).

| systemic | topical | intraocular | vaccines |
|---|---|---|---|
| • TNF-α inhibitors<br>• rifabutin<br>• bisphosphonates<br>• sulfonamides<br>• diethylcarbamazine<br>• fluoroquinolones<br>• oral contraceptives<br>• topiramate<br>• trifluoperazine<br>• quinidine<br>• ibuprofen*<br>• reserpine<br>• sildenafil<br>• clomiphene<br>• immune checkpoint inhibitors (ipilimumab, nivolumab, pembroluzimab) | • prostaglandin analogs<br>• metipranolol<br>• corticosteroids<br>• cholinomimetics<br>• brimonidine<br>• antibiotics<br>• betaxolol<br>• cholinesterase inhibitors | • antibiotics<br>• cidofovir<br>• urokinase<br>• plasmin - microplasmins<br>• monoclonal antibodies (e.g. ranibizumab, bevacizumab)<br>• triamcinolone acetonide | • Bacille Calmette-Guérin (BCG)<br>• influenza<br>• hepatitis B<br>• measles, mumps, rubella (MMR)<br>• diphtheria, tetanus, and pertussis (dpt)<br>• varicella<br>• smallpox<br>• SARS-CoV-19-Astra-Zeneca brand |

*Table 1.3:A summary of medications associated with uveitis by type, from studies (124, 125)*
**through a condition called tubointerstitial nephritis and uveitis (TINU).*
*[+]Other causes that have emerged include COVID vaccination related uveitis, which presents with either anterior or intermediate uveitis and can occur 2-3 weeks following the vaccination. The effect is more marked in DNA Vaccinations such as the Astra Zeneca Vaccine (126).*

Tattoo and eyeliner related uveitis is thought to be a granulomatous process associated with the ink in tattoos and other skin altering procedures (41, 127).

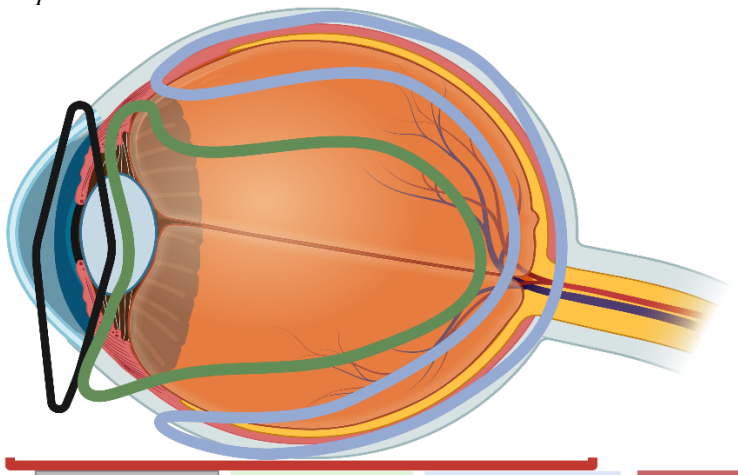
Below are a table and graph combining the aetiology and most likely cause by location of uveitis (table 1.4 and figure 1.6).

| | anterior | intermediate | posterior | panuveitis |
|---|---|---|---|---|
| **Infectious:** | | | | |
| HSV | +++ (uni>bilateral) | 0 | ++ (diffuse choroiditis) | ++ |
| Tuberculosis | + (granulomatous chronic) | 0 | +++ (diffuse choroiditis) | 0 |
| Syphilis | ++ (granulomatous chronic) | + | +++ | ++ |
| Toxoplasmosis | | 0 | ++ (focal chorioretinitis) | ++ |
| Bartonella | | | | |
| HIV | | 0 | | |
| CMV | ++ (bilateral) | 0 | | ++ |
| VZV | ++ (bilateral) | 0 | | ++ |
| Lyme Disease | 0 | ++ | 0 | 0 |
| **immunological:** | | | | |
| demyelination related (Multiple sclerosis) | 0 | +++ | ++ (retinal vasculitis) | 0 |
| MOGAD and NMOSD | + | ++ (bilateral) | ++ | ++ |
| COVID vaccine related | ++ | +++ | ++ | +++ |
| sarcoid | ++ (chronic granulomatous) | ++ | | +++ |
| Bechet's disease | + | ++ | + | ++ |
| Kawasaki disease | ++ | 0 | 0 | 0 |

| | | | | |
|---|---|---|---|---|
| post-streptocococcal | ++ (acute bilateral) | 0 | 0 | 0 |
| TINU | + (acute bilateral) | 0 | 0 | 0 |
| **genetic/haplotype related:** | | | | |
| HLA-B27 related (includes IBD, reactive arthritis, PsA, ank. spond.) | +++ (acute unilateral/bilateral) (uni>bilateral) | 0 | 0 | 0 |
| Fuchs cyclitis | ++ (chronic non granulomatous) | ++ | + | 0 |
| Vogt-Koyanagi-Harada disease | 0 | ++ | ++ (diffuse choroiditis | +++ |
| **medication/toxins:** | | | | |
| rifabutin/bisphosphonates | +++ (bilateral) | 0 | 0 | 0 |
| TNF-α inhibitors | | | | |
| checkpoint inhibitors mercury | | | | |
| **other causes:** | | | | |
| Birdshot Chorioretinopathy | | | +++ (diffuse chorioiditis) | |
| lymphoma skin related/ink | | ++ (if over 40) | | |

*Table 1.4: Classification of causes of uveitis by location and likelihood of location. Table illustrating the etiologies and the likelihood by location by aetiology. Some aetiologies only mentioned in table. (5, 18,23). Abbreviations: HSV: Herpes Simplex Virus, HIV: Human Immunodeficiency Virus, CMV: Cytomegalovirus, VZV: Varicella-Zoster Virus, MOGAD: Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease,*

*NMOSD: Neuromyelitis Optica Spectrum Disorder, TINU: Tubulointerstitial Nephritis and Uveitis, IBD: Inflammatory Bowel Disease, PsA: Psoriatic Arthritis, TNF-α: Tumor Necrosis Factor alpha*



| | Anterior Uveitis Iris and Ciliary Body | Intermediate Uveitis (pars plana and vitreous | Posterior Uveitis (Choroid +Retinitis, Neuroretinitis) | Panuveitis (all structures |
|---|---|---|---|---|
| **Infectious** | HSV(most common)<br>Syphilis<br>CMV<br>VZV | Syphilis<br>Lyme disease | VZV, Bartonella<br>Syphilis ,Toxoplasmosis<br>CMV, Candida, West Nile,<br>Tuberculosis | Syphilis<br>Lyme disease<br>Tuberculosis |
| **Immune** | HLAB-27 associated<br>JIA associated<br>Sarcoidosis<br>Behcet's Disease | MS-associated Uveitis<br>Sarcoidosis | Sarcoidosis | Vogt-Koyanagi- Harada disase<br>Behcet's disease<br>Sarcoidosis |
| **Syndromic** | Fuch's uveitis | Pars Planitis | Birdshot Chorioretinitis<br>Relentless placoid<br>PIC<br>MFCPU<br>MWEDS<br>APMPPE | Sympathetic ophthalmia |

*Figure Table 1.5. Anatomical classification of uveitis.* Anatomical classification of uveitis and regions affected – anterior (grey), intermediate (green), posterior (blue), panuveitis (pink). APMPPE (Acute Posterior Multifocal Placoid Pigment Epitheliopathy), CMV (Cytomegalovirus), HSV (Herpes Simplex Virus), JIA (Juvenile Idiopathic Arthritis), MFCPU (Multifocal Choroiditis and Panuveitis), MS (Multiple Sclerosis), MWEDS (Multiple Evanescent White Dot Syndrome), PIC (Punctate Inner Choroidopathy), VZV (Varicella-Zoster Virus) From (128-132)

### 1.6.4 Idiopathic uveitis

Idiopathic uveitis refers to uveitis in which no clear aetiology has been established after investigation. This can occur in up to a third of patients (33). Idiopathic uveitis is one the most common uveitis diagnoses in Europe and the United States (133). These patients are often subjected to prolonged or repeated courses of corticosteroids, which can improve their symptoms and visual parameters, but responses may only be temporary. However, corticosteroids hold risks of their own, including an increased risk of ocular or systemic infection, cataracts and other metabolic and bone health adverse effects (134). 60-85% of patients with idiopathic uveitis in a different case series maintain a visual acuity of 20/40 or better (32). Other studies suggest that early treatment and lack of activity at 3 months, as well as younger age at onset is associated with fewer complications in idiopathic uveitis (135).

Follow-up is important in idiopathic uveitis to ensure the clinical diagnosis remains idiopathic and no other cause is found. 29% of patients with idiopathic uveitis patients in tertiary centre had a systemic diagnosis identified in a series of 179 over a course of 8 year, including sarcoidosis, HLA-B27 associated uveitis, tubular interstitial nephritis with uveitis and other (136).

Finally, new studies are using predictive artificial intelligence (AI) and genomic and transcriptomic testing to help identify subsets of idiopathic uveitis and define new syndromes. Looking at transcriptomics of patients with idiopathic compared to four distinct syndromes of uveitis, helped reclassify 11 out of 38 patients into the other subgroups (e.g. sarcoidosis) (137, 138).

## 1.7 Clinical complications of uveitis

There are ophthalmologic complications that can occur in untreated or undertreated uveitis. These include macular oedema, cataracts, glaucoma, synechiae, band keratopathy, vitreous opacities, vitreous haemorrhage, retinal neovascularisation, and retinal detachment (134) (Table 1.5). All these complications can have effects on visual acuity and ultimately lead to blindness (11, 96). Non-ophthalmic complications of uveitis include ongoing pain and headaches (139).

| Characteristic | Definition |
|---|---|
| Cystoid Macular Edema (CME) | Most common cause of vision loss due to chronic fluid accumulation in the macula. |
| Cataracts | Secondary to chronic inflammation or prolonged corticosteroid therapy. |
| Glaucoma/Ocular Hypertension | Elevated intraocular pressure from trabecular meshwork damage or steroid use. |
| Retinal Detachment | May occur due to vitreous traction or exudative retinal detachment in severe cases. |
| Chorioretinal Scarring & Atrophy | Irreversible damage from prolonged inflammation, leading to permanent vision impairment. |
| Band Keratopathy & Hypotony | Advanced cases may develop corneal calcification (band keratopathy) or phthisis bulbi (shrunken eye). |
| Cystoid Macular Edema (CME) | Most common cause of vision loss due to chronic fluid accumulation in the macula. |

*Table 1.6. List of complications of uveitis and associated changes (140-142).*

## 1.8 Classification of the course of uveitis

Uveitis can be classified in terms of acuity and disease course. Uveitis is classified as acute from onset to a period of three months. It is classified as recurrent if it happens twice or more within a

duration greater than three months. Finally, it is said to be chronic if an episode lasts for more than three months. The characterisation of acuity and course can aid with diagnosis (134).

### 1.8.1 Classification of severity

As part of the consensus classification, the severity of uveitis is quantified based on clinical appearances (see table 1.6 and table 1.7 for anterior chamber classification). This classification relies on the presence of cells and characterisation of the vitreous fluid as observed on the slit-lamp examination, as well as the retinal descriptions and the optic disc swelling. Disease severity influences therapeutic pathways (33).

| score | description | clinical findings |
|---|---|---|
| 0 | nil cells | nil |
| 0.5 | trace | nil |
| 1 | minimal | posterior pole visible |
| 2 | mild | posterior pole slightly hazy |
| 3 | moderate | posterior pole very hazy |
| 4 | marked | posterior pole barely visible |
| 5 | severe | fundus details not visible |

*Table 1.7: The severity of anterior and posterior chamber uveitis (46).*

| grade/description of anterior chamber (AC) flare | grade of AC cells | number of cells in field (1x1mm slit beam) |
|---|---|---|
| 0-none | 0- | <1 |
| | 0.5+ | 1-5 |
| 1+ faint | 1+ | 6-15 |
| 2+ moderate (iris and lens details clear) | 2+ | 16-25 |
| 3+ marked (iris and lens details hazy) | 3+ | 26-50 |
| 4+ intense (fixed and plastic aqueous) | 4+ | 50+ |

*Table 1.8: Clinical classification of severity of anterior chamber. From SUN 2005 classification. The consensus classification of the activity in the anterior chamber (33).*

## 1.9 Overview of the immune system in the eye

The eye is thought to be immune privileged, whereby the immune system does not mount a typical immune response in order to protect the organ from damage by inflammatory responses (143). The term was coined by Sir Peter Medawar in the 1940s when evaluating immune privilege in the context of transplant medicine, whereby transplanting other body tissue into the anterior chamber of the eye did not elicit an immune response (144). Immune privilege in the eye is supported by the fact that 90% of corneal transplants do not experience rejection (145).

There are a few mechanisms that may contribute to immune privilege (146). Firstly, physical barriers exist which provide a separation. This is specifically defined by the blood retina barrier (BRB) by way of tight junctions in the retinal pigment epithelial layer ( see figure 1.7) and lack of exiting lymphatics (147). The BRB regulates the influx and the exit of micronutrients and substances. Sources of regulation include the tight junctions previously mentioned, as well as caveolae , which cave-like invaginations which trap large molecules (148), and Muller cells which create the foot processes (147) (Figure 1.7).

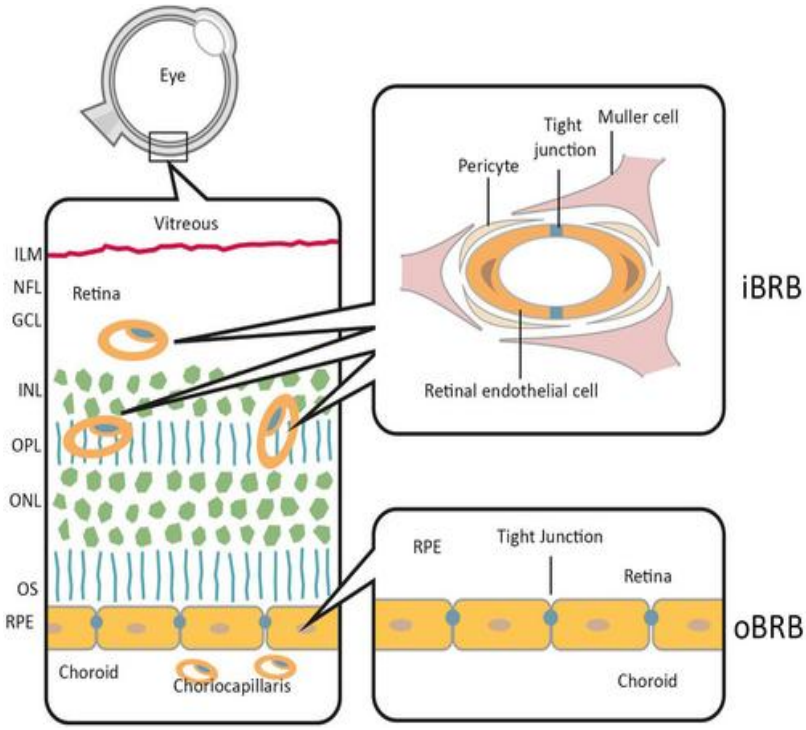



*Figure 1.7: Localisation of the inner and outer BRB. The iBRB is formed by endothelial cells lining the vasculature of the inner retina. These vessels vascularise the retina up to and including the OPL, with the photoreceptor cell layer remaining avascular. The oBRB is formed by the retinal pigment epithelial cells (RPE) and regulates the exchange of material from retina to choroid. Abbreviations: blood retina barrier (BRB), inner limiting membrane (ILM), ganglion cell layer (GCL), inner nuclear layer (INL), outer nuclear layer (ONL) and outer segments (OS). From (147).*

Secondly, an anti-inflammatory milieu in the eye exists, which is made up of soluble and insoluble regulatory compounds. These include tumour growth factor (TGF)-β, α-melanocyte stimulating hormone, and vasoactive intestine peptide. The Muller cells and the pigmented epithelium in the retina, iris and ciliary body, inhibit T cells and induce cluster of differentiation (CD)86, CTLA-4 (clathrin light-chain A) and Fas ligand (FasL). These chemokines induce Tregulatory to further tighten the junction and inhibit further immune active cells from entering the retina (149).

Anterior chamber associated immune deviation (ACAID) refers to the induction of a systemic form of tolerance to an external antigen introduced into the ocular environment in order to prevent damage to the ocular structures (149). The concept of “immune privilege” is based on the

expression of immunosuppressive factors on ocular tissue and fluids that can suppress immune responses that may otherwise result in damage to ocular tissues. The induction of ACAID occurs due to infiltration of circulating monocytes that then emigrate to the thymus and spleen where they induce T regulatory cells that inhibit the escalation of a cell-mediated immune response (Figure 1.8 from(150)). The immune system of the eye can also induce apoptosis of infiltrating immune cells by FasL expression, as well as by selectively activating a Th 2 (T-helper cell type 2) population, thereby further reducing immune/inflammatory damage to the eye (151, 152).

The study by Stein-Streilein (149), highlights how the eye employs mechanisms like anterior chamber-associated immune deviation (ACAID) to suppress inflammation and maintain tolerance. However, dysregulation of these processes can lead to autoimmune conditions such as uveitis. The paper emphasizes the roles of regulatory T cells and cytokines in balancing immune responses, preventing excessive inflammation while protecting against pathogens. It also discusses how breakdowns in immune tolerance can trigger uveitis, underscoring the importance of understanding ocular immune regulation for developing targeted therapies. This work provides a foundation for exploring immune-mediated pathways in the pathogenesis of uveitis.

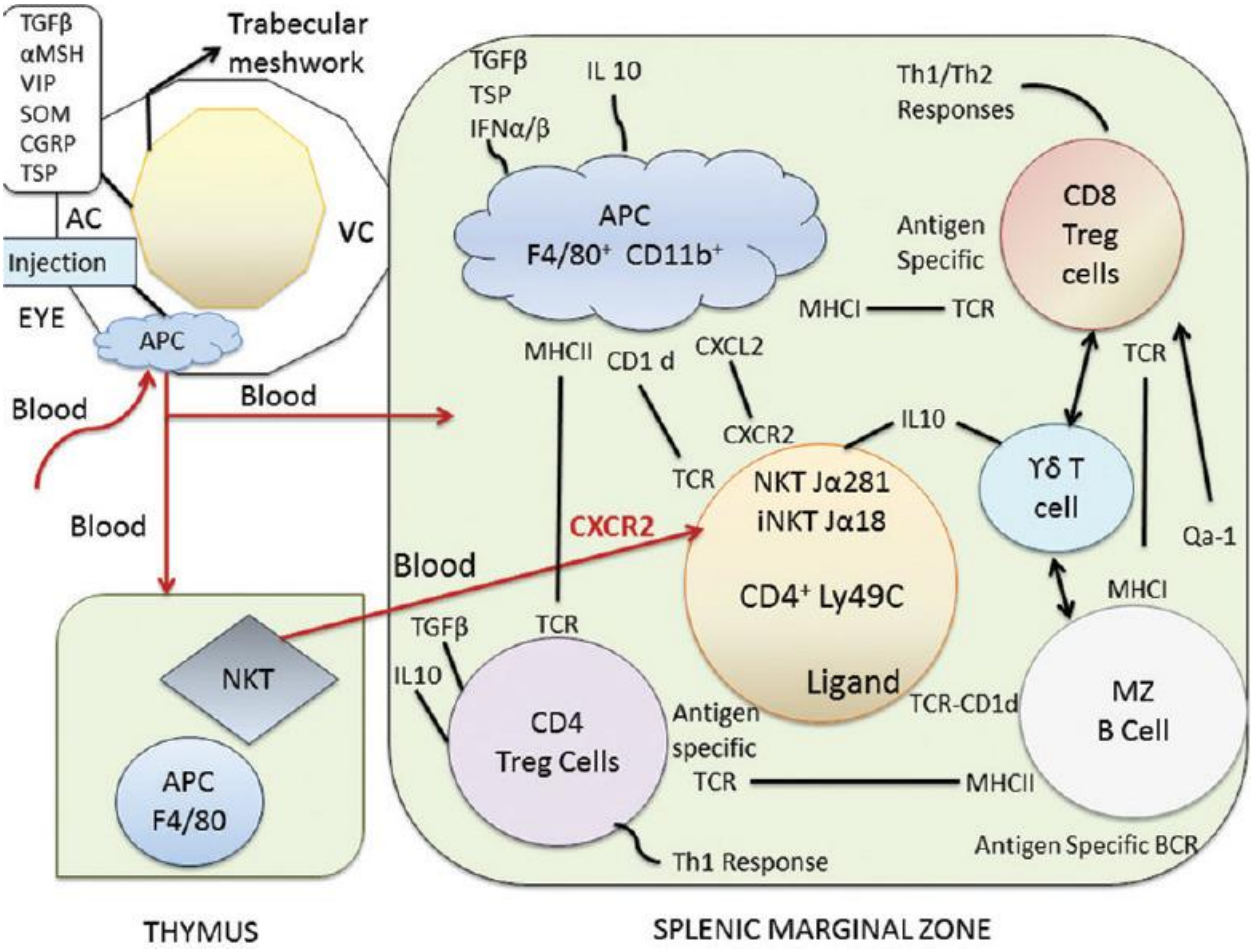



*Figure 1.8: Anterior chamber associated immune deviation is a mult-organ generalized process. Anterior chamber associated immune deviation is the presence of a highly immune mute milieu in the uvea causing the release of inhibitory cytokines in the periphery which causes these tissues to release regulatory molecules such as IL-10 and FAS and FAS-L Abbreviations: IL-10: interleukin 10, Fas-L: Fas Ligand (137).*

### 1.9.1 Cytokines as a marker of different manifestations of disease

Cytokines are molecules secreted by the immune system cells in response to an immunogenic stimulus (153). Cytokines primarily enable communication between appropriate immune cells and serve to escalate or diminish immune responses and inflammation accordingly. Of these cytokines, a subgroup of important molecules is called chemokines, which are small molecules whose main

function is to facilitate movement of molecules in the immune system (154). Cytokines have multiple functions including:

1. Signalling: differentiation of certain types of immune cells and increasing/decreasing the response of particular subsets of immune cells
2. Facilitating an inflammatory or anti-inflammatory response
3. Regulation of immune responses based on feedback loops
4. Increasing permeability and ability of immune cells to move to the circulation via cell adhesion molecules
5. Chemokines: serve to direct chemicals or immune cells towards tissue injury (153).

| **Proinflammatory cytokines** | | |
|---|---|---|
| **Cytokine** | **Principal source** | **Primary activity** |
| **GM-CSF** | Th cells | Growth and differentiation of monocytes and dendritic cells |
| **IL-1α** | Macrophages and other APCs | Costimulation of APCs and T cells, inflammation and fever, acute phase response, hematopoiesis |
| **IL-1β** | Macrophages and other APCs | Costimulation of APCs and T cells, inflammation and fever, acute phase response, hematopoiesis |
| **IL-2** | Activated Th1 cells, NK cells | Proliferation of B cells and activated T cells, NK functions |
| **IL-3** | Activated T cells | Growth of hematopoietic progenitor cells |
| **IL-5** | Th2 and mast cells | Eosinophil growth and function |
| **IL-6** | Activated Th2 cells, APCs, other somatic cells | Acute phase response, B cell proliferation, thrombopoiesis, synergistic with IL-1 and TNF on T cells |
| **IL-7** | Thymic and marrow stromal cells | T and B lymphopoiesis |
| **IL-8** | Macrophages, somatic cells | Chemoattractant for neutrophils and T cells |
| **IL-9** | T cells | Hematopoietic and thymopoietic effects |
| **IL-12** | B cells, macrophages | Proliferation of NK cells, IFN production, promotes cell-mediated immune functions |
| **IL-18** | Macrophages | Potent inducer of IFN-γ by T cells and NK cells |
| **IFN-α** | Macrophages, neutrophils and some somatic cells | Antiviral effects, induction of class I MHC on all somatic cells, activation of NK cells and macrophages |
| **IFN-γ** | Activated Th1 and NK cells | Induces of class I MHC on all somatic cells, induces class II MHC on APCs and somatic cells, activates |

| | | |
|---|---|---|
| | | macrophages, neutrophils, NK cells, promotes cell-mediated immunity, antiviral effects |
| **MIP-1α** | Macrophages | Chemotaxis |
| **MIP-1β** | Lymphocytes | Chemotaxis |
| **TNF-α** | Macrophages, mast cells, NK cells, sensory neurons | Cell death, inflammation, pain |

*Table 1.8a is for proinflammatory cytokines. Adapted from(155-162). Abbreviations: APC: antigen presenting cell, MHC: major histocompatibility complex, Ig: immunoglobulin, NK: natural killer, GM-CSF: granulocyte-macrophage colony stimulating factor, IL: interleukin, IFN: interferon, MIP: macrophage inflammatory protein, TNF: tumour necrosis factor.*

| **Anti-inflammatory cytokines** | | |
|---|---|---|
| **Cytokine** | **Principal source** | **Primary activity** |
| **IFN-β** | Macrophages, neutrophils and some somatic cells | Antiviral effects, induction of class I MHC on all somatic cells, activation of NK cells and macrophages |
| **TGF-β** | T cells, monocytes | Chemotaxis, IL-1 synthesis, IgA synthesis, inhibit proliferation |
| **IL-4** | Activated T cells | B cell proliferation, eosinophil and mast cell growth and function, IgE and class II MHC expression on B cells, inhibition of monokine production |
| **IL-10** | Activated Th2 cells, CD8+ T and B cells, macrophages | Inhibits cytokine production, promotes B cell proliferation and antibody production, suppresses cellular immunity, mast cell growth |
| **IL-11** | Stromal cells | Synergistic hematopoietic and thrombopoietic effects |
| **IL-13** | Th2 cells | IL-4-like activities |
| **TNF-β** | Th1 and Tc cells | Phagocytosis, Nitrous Oxide production, cell death |

*Table 1.8b anti-inflammatory cytokines. Adapted from (155-162). Abbreviations: APC: antigen presenting cell, MHC: major histocompatibility complex, Ig: immunoglobulin, TNF: tumour necrosis factor, TGF: tumour growth factor, CD: cluster of differentiation*

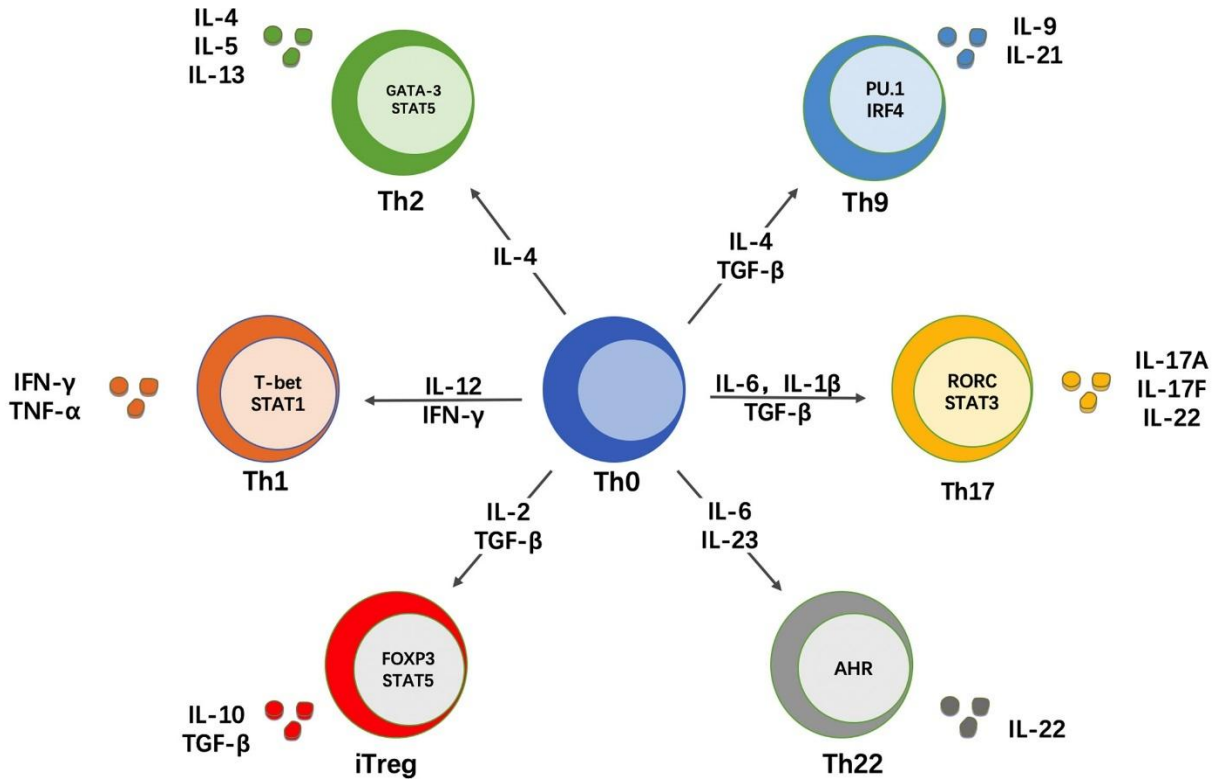



*Figure 1.9: The differentiation of a naïve T cell. An illustrative figure showing the various pathways an immature T-cell can differentiate into, depending on the cytokines it encounters and the cytokines it subsequently produces. From (163). Abbreviations: Th: T-helper cell, IL: interleukin, TGF: tumour growth factor, GATA and STAT are genes and do not stand for anything: , IFN: interfereon, Treg: T-regulatory cell, FOXP3: forkhead box protein 3, AHR: aryl hydrocarbon receptor, RORC: RAR-related orphan receptor C.*

The role of cytokines in differentiating infectious from immune pathologies is evident in many types of neuroinflammation. The disease state can dictate which type of cell the naïve t-cell differentiates to (figure 1.9). A meta-analysis in 2016 (147), evaluated different cytokines in the cerebrospinal fluid (CSF) of patients with inflammatory central nervous system pathologies. The study found that C-X-C motif chemokine ligand 13 (CXCL13) and B-cell activating factor (BAFF) were elevated in antibody mediated disorders. IFN-γ: Interferon gamma was elevated mainly in viral encephalitis. Th-2 and Th-17 was increased in acute disseminating encephalomyelitis and neuromyelitis optical spectrum disorder in comparison to MS, where Th-1 and Th-17 are more

elevated (164). Another study by the same group looked at cytokines in N-methyl-D-aspartate receptor (NMDAR) encephalitis versus enterovirus encephalitis and found highly sensitive cytokines in close to 80% of samples. CSF Th-17 and Th-2-related cytokines were more correlated with NMDAR encephalitis. Furthermore, CXCL13 and CXCL10 were associated with worse outcomes (165, 166). These studies postulate that serum and CSF cytokines could be used to predict diagnosis in undifferentiated clinical presentations.

Cytokines play a pivotal role in the immune response and inflammation, and their profile can aid in differentiating the various causes of uveitis. Furthermore, they can be used to understand the dominant underlying pathogenic disease processes in certain disorders (see tables 1.8a and 1.8b). In uveitis, evaluating cytokine profiles in serum, aqueous humour, or vitreous humour at different stages of disease activity may facilitate diagnosis (167).

Interleukin-6 (IL-6) is a cytokine that has been implicated in the pathogenesis of uveitis, and elevated levels may be associated with non-infectious uveitis in various body fluid areas, such as autoimmune uveitis (168). In particular, the Il-10/IL-6 ratio in the vitreous humour has been identified in potentially differentiating an inflammatory or infective process from a lymphoproliferative process, whereby a ratio of IL-10/IL-6 of <1 favours inflammation as the cause of uveitis (150). Tumour necrosis factor-alpha (TNF-α) is another key cytokine that contributes to inflammation and may be elevated in both infectious and non-infectious uveitis. In particular, TNF-α in the aqueous humour has been seen in conditions like HLA-B27-associated anterior uveitis and Behçet's disease (95, 169). Interferon-gamma sampled from in intraocular

samples, produced by T-helper 1 cells, may be linked to infectious uveitis, including toxoplasmosis and tuberculosis associated uveitis, and other intracellular pathogens (170).

Th1-mediated responses may be associated with infectious uveitis, while Th2 responses are implicated in autoimmune uveitis (163). Within infective uveitis, there may be further differentiating cytokine profiles. For example, the cytokine profile of Toxoplasma reveals elevated IL-17 compared to viral causes (170).

A study examining sera and aqueous humours from 75 patients with idiopathic uveitis, compared to controls with infectious uveitis, immune uveitis, and non-inflammatory aetiologies, revealed distinct clusters of cytokines associated with idiopathic uveitis. These cytokines include IL-1Rα, IL-6, IL-8, IL-12, IL-17, IP-10, MIP (macrophage inflammatory protein)-1α, MIP-1β, MCP (monocyte chemoattractant protein)-1, G-CSF, and TNF-α. Further analysis indicated that IL-10, IL-17, and IL-21 were specifically associated with idiopathic uveitis compared to all control groups. Additionally, cytokines such as IL-5 and IL-9 were elevated across all inflammatory groups, correlating with Th-1 and Th-9 responses previously reported in the literature (171).

In a study by Sauer et al. (170), a cytokine profile multiplex assay was used to evaluate 27 cytokines and chemokines in aqueous humour samples from patients, aiding in the diagnosis within the research cohort. This approach significantly reduced the percentage of idiopathic uveitis cases from 40% to 18% (from 25 patients to 11 patients), ultimately guiding more accurate treatment plans. This highlights the utility of cytokine profiling from intraocular fluid in differentiating uveitis aetiologies and improving diagnostic precision.

The research by Sauer et al. (2015) and Errera et al. (2022) (171) adds substantial evidence to the field of cytokine profiling in uveitis. Sauer et al. identified IL-17A and IL-10 as markers for toxoplasmosis and viral uveitis, respectively, through intraocular levels, while Errera et al. provided a comprehensive profile of cytokines, chemokines, and growth factors in human aqueous humour in idiopathic uveitis.

Below is a summary of the common changes in cytokines in certain aetiologies of uveitis (Table 1.9). Table 1.10 describes the increase in serum or aqueous or vitreous humour cytokines in certain conditions.

| Disease (ocular fluid) | Increase | Decrease | No Change or Not Detectable |
|---|---|---|---|
| Immune Pathologies | | | |
| Behçet disease (aqueous) | TNF-α, IL-1β, IL-6, IL-8, CCL2, IL-22, IP-10, IL-12 | TNF-α, IL-1β, IL-6, IL-17A, CCL2 | TNF-α, IL-1β, IL-6, IL-17A, CCL2 |
| Birdshot chorioretinopathy (aqueous) | TNF-α, IL-1β, IL-6 | IL-8, CCL2 | - |
| HLA-B27-positive uveitis (aqueous) | TNF-α, IL-1β, IL-8, IL-6 recently | - | CCL2 |
| Juvenile idiopathic arthritis (aqueous) | IL-6, IL-8 | - | CCL2 |
| Sarcoid uveitis (aqueous) | TNF-α, IL-1β, IL-8,eotaxin | - | CCL2 |
| Sarcoid uveitis (vitreous) | TNF-α, IL-6, IL-8, IL-17A, CCL2 | - | IL-1β |
| Vogt-Koyanagi-Harada syndrome (aqueous) | TNF-α, IL-1β, IL-6, IL-8, CCL2 | TNF-α, IL-1β, IL-6, IL-8, IL-17A, CCL2 | TNF-α, IL-1β, IL-6, IL-8, IL-17A, CCL2 |
| Demyelinating Autoimmune including NMDA receptor antibody disease and experimental autoimmune disease (CSF and serum) | Th17, IL-17, IL-21,IL-4, CCL-17, CXCL-13/10 | - | - |

| | | | |
|---|---|---|---|
| Fuch's iridocyclitis (aqueous) | IL-8, IFN-gamma, MCP-1,MIP-1β, TNF-α | - | - |
| Idiopathic Pathologies | | | |
| Idiopathic uveitis (serum and aqueous) | TNF-α, IL-6, IL-7, IL-8, IL-9, CCL2, IP-10, IL-17, IL-21, IL-23, IL-Ra, G-CSF, MCP-1, MIP-1α, RANTES, VEGF | TNF-α, IL-1β, IL-6, IL-17A, CCL2, IFN-gamma, IL-5 | TNF-α, IL-1β, IL-6, IL-17A, CCL2 |
| Infectious Pathologies | | | |
| Viral uveitis (serum and aqueous) | IL-1β, IL-2, IL-15,IFN-gamma, IL-12, IL-6, IL-4, IL-5, IL-13, IL-10, IL-8, MIP-1β, MIP-1α, MCP-1, IP-10,G-CSF,RANTES, Eotaxin, VEGF, PDGF-BB | - | - |
| Toxoplasma uveitis (serum and aqueous humour) | IL-12, Il-17, G-CSF, IFN-gamma, IP-10,eotaxine, MCP-1 TNF- α, IL-5, IL-6, IFN-γ, IL-15, MIP-1β, MIP-1α, MCP-1, IP-10, RANTES, Eotaxin, PDGF-BB | - | - |
| Tuberculous Uveitis Vitreous and serum samples | IFN-γ, TNF-α, IL-6, and IL-10, CCL-2, CXCL8, CXCL9, CXCL10 | IL-9 | - |
| Syphilitic uveitis Aqueous and vitreous samples | IL-17F, TNF RI, TNF RII, IL-16, OPN, and MCSFR | - | MCP-3, LIF, G-CSF, MIP-3a, and GH |

*Table1.9: Cytokines in the literature in different studies including type of specimen collected (aqueous, CSF, serum) and aetiology* (95, 150, 169, 171-174)

| | **Elevated serum levels** | **Elevated aqueous humour levels** | **Elevated vitreous levels** |
|---|---|---|---|
| IL-1 | HLA-B27-Associated Uveitis | HLA-B27-Associated Uveitis, Birdshot Chorioretinopathy | Sarcoid uveitis |
| IL-2 | Idiopathic Pediatric Uveitis, Bechet's Disease, FUS | HLA-B27-Associated Uveitis, Idiopathic Anterior Uveitis, Fuchs' Uveitis syndrome ,Juvenile Idiopathic Arthritis Uveitis, Birdshot Chorioretinopathy, Bechet's Disease | Idiopathic Pediatric Uveitis |
| IL-6 | HLA-B27-Associated Uveitis, Fuchs' Uveitis syndrome, Bechet's Disease | HLA-B27- Associated Uveitis, Idiopathic Anterior Uveitis, Birdshot Chorioretinopathy, Bechet's Disease, Vogt-Koyanagi-Harada | HLA-B27-AU, Fuchs' Uveitis syndrome, Juvenile Idiopathic Arthritis Uveitis, Sarcoidosis, Pars planitis, Multiple Sclerosis, Idiopathic Pediatric Uveitis, Bechet's Disease |
| IL-15 | | HLA-B27- Associated Uveitis, Bechet's Disease, Vogt-Koyanagi-Harada | |
| IFN-γ | Bechet's Disease, Sarcoid uveitis | HLA-B27-associated disaease, Idiopathic Anterior Uveitis, Fuchs' Uveitis Syndrome, Juvenile Idiopathic Arthritis Uveitis, Behcet's Disease, Vogt-Koyanagi-Harada | Sarcoid uveitis |
| TNF-α | Bechet's Disease | HLA-B27-Associated Uveitis, Fuchs' Uveitis Syndrome, Juvenile Idiopathic Arthritis Uveitis, Birdshot Chorioretinopathy, Vogt-Koyanagi-Harada, Bechet's Disease | Sarcoid uveitis |

| | | | |
|---|---|---|---|
| IL-17 | Sarcoid uveitis, Idiopathic Anterior Uveitis, Idiopathic Pediatric Uveitis, Birdshot Chorioretinopathy , Vogt-Koyanagi-Harada, Bechet's Disease | HLA-B27- associated Uveitis, Idiopathic Anterior Uveitis, Birdshot Chorioretinopathy, Bechet's Disease Vogt-Koyanagi-Harada | - |
| IL-23 | Birdshot Chorioretinopathy Bechet's Disease, Vogt-Koyanagi-Harada | - | - |
| IL-8 | - | Idiopathic Anterior Uveitis, Bechet's Disease, Vogt-Koyanagi-Harada | Sarcoid uveitis |
| MCP-1 | - | Fuchs' Uveitis Syndrome | |
| MIP-1β | - | Fuchs' Uveitis Syndrome | Sarcoid uveitis |
| IL-10 | Elevated intraocular levels are attributed to regulatory mechanisms activated along with inflammation. IL-10/IL-6 <1; suggestive of uveitis IL-10/IL-6 >1; suggestive of intraocular lymphoma | | |

*Table 1.10: Association of certain cytokines with certain aetiologies group by cytokine. From (150).*

Evaluating acute serum cytokine levels, along with other clinical and laboratory parameters, could contribute to a more precise diagnosis and guide the selection of appropriate therapeutic interventions in uveitis conditions (175).

## 1.10 Current unmet needs

There is currently a dearth of literature related to non-invasive investigations that may help differentiate infectious from immune-mediated causes of uveitis. Furthermore, there is a lack of biomarkers to classify patients with idiopathic uveitis, and these patients risk delayed diagnosis

and treatment, resulting in worse outcomes. Current studies evaluating this question are often retrospective, may include patients with chronic uveitis, or require invasive investigations such as intraocular sampling.

Our study builds on these findings by focusing on non-invasive serum samples and expanding the sample size to include acute-phase samples. This approach aims to refine the differentiation of infectious versus immune-mediated uveitis, utilising cytokine profiles to classify undifferentiated cases more accurately and guide targeted treatments.

## 1.11 Aims

**Aim 1:** To prospectively evaluate a cohort of patients with acute uveitis in order to delineate the natural history, clinical presentations, therapeutic responses, and outcome measures of patients with confirmed infectious versus immune-mediated uveitis and compare this to patients with idiopathic uveitis.

**Aim 2:** To identify clinical and immunological biomarkers which may differentiate immune-mediated from infectious aetiologies, via non-invasive profiling including clinical and serological biomarkers.

**Aim 3:** To apply any identified clinical and immunological biomarkers to better classify patients with idiopathic uveitis.

**Aim 4:** To evaluate the value of clinical and immunological biomarkers to predict severity, therapeutic responses, and outcomes in immune-mediated, infectious and idiopathic uveitis.

## 1.12 Hypotheses

**Hypothesis 1:** There will be immunological biomarkers which help differentiate patients with uveitis from healthy controls.

**Hypothesis 2:** There will be clinical and/or immunological biomarkers which help differentiate immune mediated uveitis from infectious uveitis.

**Hypothesis 3:** Identifying these differentiating profiles may assist with better classifying patients with idiopathic uveitis, with therapeutic and prognostic implications.

## 1.13 Significance and outcomes

This study will focus on the comprehensive prospective evaluation of a cohort of uveitis patients, with acute non-invasive biological samples obtained within three months of onset or relapse of uveitis. This study will enable the identification of clinical and immunological profiling as a valuable tool in the diagnosis and management of uveitis.

# Chapter 2 Methods

## 2.1 Participant recruitment and inclusion and exclusion criteria

### 2.1.1 Participant Recruitment

This study obtained comprehensive clinical information in patients with acute uveitis presenting to eye clinics in two tertiary hospitals in New South Wales, Australia - Westmead Hospital and Sydney Eye Hospital. All patients provided written informed consent.

Data was collected from the following sources:

1. Structured interviews with patients during their initial clinical evaluation in person where possible (or via a phone interview during a period of COVID restrictions for face-to-face consultations).
2. Detailed analysis of clinical notes inclusive of paper files and electronic medical records.
3. Evaluation of visual fields, optical coherence tomography (OCT), and fluorescein angiography as outlined in section 2.3.
4. Evaluation of all available infectious and immune blood tests and radiology undertaken as outlined in sections 2.4 and 2.5.
5. Serological evaluation of cytokine and autoantibody profiles as outlined in section 2.6.

### 2.1.2 Inclusion criteria

All consecutive patients from the two uveitis clinics were screened from July 2021 to February 2023. Inclusion criteria were:

-Confirmed diagnosis of uveitis (of any aetiology).

-Acute presentation of uveitis with consent and sample collection obtained within 90 days of clinical onset, inclusive of onset presentation or a relapse of known uveitis.

### 2.1.3 Exclusion criteria

-Patients outside the 90-day timeframe or classified under a chronic condition.

-Diagnosis not consistent with uveitis.

-Patient who was not willing to provide written consent

## 2.2 Clinical phenotyping

The information collected from the participants in the study is outlined in the next sections.

### 2.2.1 Demographic information

Patients were interviewed with clinical information obtained including description and chronology of symptoms, exposure to medications or chemicals that might cause uveitis, description of previous diagnoses related to uveitis, family history of immune disease and current medications (see Table 2.1 and figure 2.1).

### 2.2.2 Symptoms and clinical assessment

Symptoms associated with uveitis were assessed. These included a description of the initial episode of uveitis, and/or previous episodes. Furthermore, other systemic symptoms, that are relevant to certain diagnoses, were ascertained including mouth ulcers, arthropathies, fevers, weight loss.

| *Category* | Data collected |
|---|---|
| *Demographics* | • Hospital clinic |
| | • Date of birth |
| | • Gender |
| | • Ethnicity |
| | • Date of symptom onset |
| | • Date of sample collection |
| | • First presentation or relapse |
| | • Medications while on sample collection |
| | • Time in days from symptoms onset to sample collection |
| *Clinical data* | • Working diagnosis of current episode |
| | • Age at symptom onset in initial episode |
| | • Age at symptom onset in current episode |
| | • Laterality |
| | • Uveitis etiology (immune, idiopathic, infectious, other) |
| | • Previous systemic diagnosis (if applicable) |
| | • Optic disc swelling |
| | • Classification of uveitis – anterior, intermediate, posterior, panuveitis |
| | • Associated optic neuritis present or absent |
| | • Associated optic perineuritis present or absent |
| | • Eye pain (scale from 0-10, including at worst ,and most recent clinic visit. Other data included does pain wake patient up night) |
| | • Description of symptoms: pain, blurriness, floaters, subjective changes, headaches, etc.<br>• Systemic symptoms: mouth ulcers, fevers, arthropathy, gut symptoms, weight loss, rashes, etc. |
| | • Family history of autoimmunity |
| | • COVID vaccinations including brand and timepoint, if available |

| | |
|---|---|
| | • Exposure to chemicals or agents related to uveitis |
| | • Medication history |
| | • Response after topical treatment |
| | • Response after oral treatment |
| | • Total number of episodes<br>• Data at follow up (further relapses, visual acuity at latest follow up) |
| | • Visual acuity at all clinic visits |
| *Ophthalmic data* | • Humphrey Visual fields at all clinic visits |
| | • Indocyanine green examinations |
| | • Fluorescein angiography examinations |
| | • Optical coherence tomography at all clinic visits: retinal nerve fibre layer measurements in micrometers, and central macular thickness |
| | • Presence or absence of macular oedema |
| | • Presence or absence of optic disc swelling based on fundoscopy exam/slit lamp exam by ophthalmologist |
| | • Other ophthalmic diagnosis before, during and after episode |
| | |
| *Radiology* | • CT Chest |
| | • PET scan |
| | • MRI- Brain or spine |
| | • CT or MRI of the orbits |
| | • Other imaging |
| | • Chest X-Ray |
| *Other investigations* | • Intraocular specimens including vitreous vs aqueous humour |
| | • Other relevant tests (e.g. MOG antibodies, AQP4 antibodies, or intrathecally restricted oligoclonal bands) |
| | • Lumbar Puncture data if any |
| *Blood tests* | • Immunological screening:<br>o Anti-nuclear antibody titre |

| | |
|---|---|
| | ○ Extractable nuclear antigen<br>○ Anti-nuclear cytoplasmic antigen<br>○ Erythrocyte sedimentation rate<br>○ C-reactive protein<br>○ Ig subclasses<br>○ Angiotensin converting enzyme<br>○ Double stranded DNA<br>○ Lupus anticoagulant<br>○ Beta-2 glycoprotein<br>○ Anticardiolipin antibody<br>○ C citrullinated protein<br>○ Rheumatoid factor<br>○ C3, C4<br>• General blood tests:<br>○ Full blood count and differentials<br>○ urea and creatinine levels<br>○ C-reactive protein<br>○ Calcium, magnesium, sodium and potassium levels<br>○ liver function tests |
| | • Infectious screening:<br>○ CMV PCR<br>○ TB IGRA i.e. Quantiferon Gold<br>○ Syphilis serology tests, VDRL, RPR confirmatory<br>○ Herpes simplex virus PCR<br>○ VZV PCR<br>○ HIV serology<br>○ Toxoplasma serology<br>○ Bartonella Serology<br>○ Chlamydia serology<br>○ Leptospira serology<br>○ Rickettsia serology<br>○ VZV PCR |

| | |
|---|---|
| | ○ HLA-B-27 |

*Table 2.1: Clinical, demographic serological and investigation data collected for our patients. Table summarising data collected for patients enrolled in study, clinical and pathological. Abbreviations: ACE (Angiotensin Converting Enzyme), ANA (Anti-Nuclear Antibody), ANCA (Anti-Nuclear Cytoplasmic Antibody), AQP4 (Aquaporin-4), C3 (Complement Component 3), C4 (Complement Component 4), CMV PCR (Cytomegalovirus Polymerase Chain Reaction), CRP (C-Reactive Protein), CT (Computed Tomography), dsDNA (Double Stranded DNA), ENA (Extractable Nuclear Antigen), ESR (Erythrocyte Sedimentation Rate), FBC (Full Blood Count), HLA-B-27 (Human Leukocyte Antigen B27), HIV (Human Immunodeficiency Virus), HSV PCR (Herpes Simplex Virus Polymerase Chain Reaction), Ig (Immunoglobulin), MOG (Myelin Oligodendrocyte Glycoprotein), MRI (Magnetic Resonance Imaging), OCT (Optical Coherence Tomography), PET (Positron Emission Tomography), RF (Rheumatoid Factor), RPR (Rapid Plasma Reagin), TB IGRA (Tuberculosis Interferon-Gamma Release Assay), VDRL (Venereal Disease Research Laboratory), VZV PCR (Varicella-Zoster Virus Polymerase Chain Reaction).*

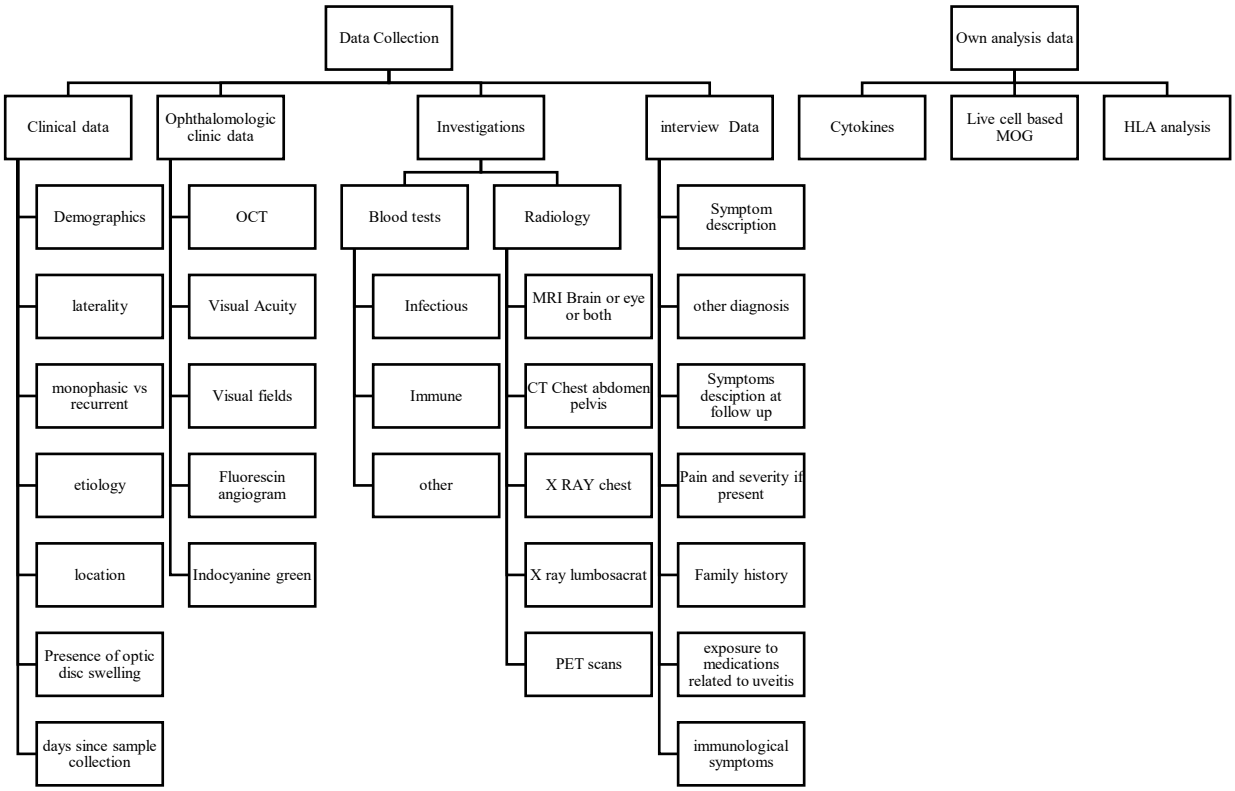



*Figure 2.1 data collected in clear form divided by category including analysed data.*

## 2.2.3 Pain evaluation

Ocular pain data was collected. This included pain at time of sample collection, date at worst eye pain, pain with eye movement and pain that wakes up patient from sleep on a validated pain scale from 0-10. Furthermore, data on headache severity and type was collected.

### 2.2.4 Therapeutic approaches

Data on the participants' medication at time of collection was collected. Furthermore, date of treatment was recorded. This included topical ophthalmic treatments, oral and infusion therapy. Information on corticosteroid treatment including dates, and dosing regimen. Furthermore, we collected data on any ophthalmological procedures.

## 2.3 Ancillary visual investigations

### 2.3.1 Visual acuity

Visual acuity was performed at every clinic visit and documented. Visual acuity was measured manually at 6 meters using Snellen charts (Figure 2.1). A visual acuity of 6/6 is considered normal and indicates what the patient can see in comparison to what a person with normal vision should be able to see at six meters distance. For stastical anylysis, the data was then LogMAR transferred. The transformation is LogMAR = -log(1/V), where "V" is the decimal visual acuity score (the lower number in the Snellen fraction). A 6/6 visual acuity corresponds to a LogMAR value of 0.0.

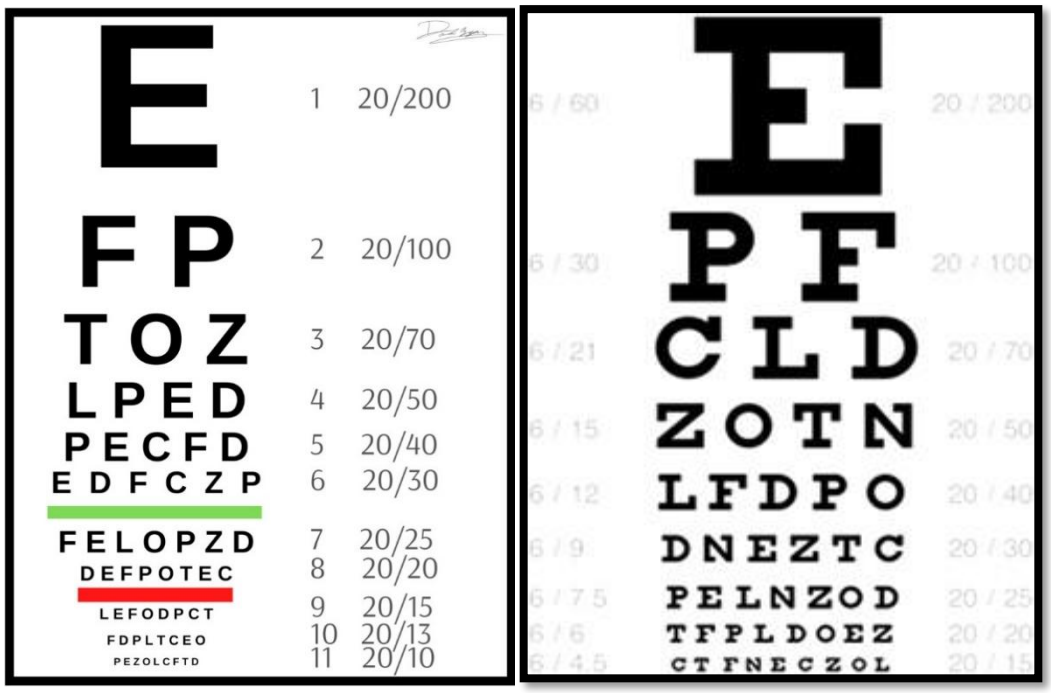



*Figure 2.2: Two different Snellen charts. An example of a Snellen Chart used to assess visual acuity usually at 6 meters or 20 feet. From (176, 177).*

### 2.3.2 Visual fields

Visual field data was evaluated using Humphrey visual fields, using a Zeiss HVF analyser (Figure 2.2). The field can be captured in 3 settings depending on the suspected pathology. 30-2 refers to a test grid that evaluates 30 degrees of the central visual field, testing 76 points spaced 6 degrees apart. It is commonly used in glaucoma and neurological diagnosis and monitoring, capturing both central and peripheral field defects. 24-2 covers 24 degrees of the central field, testing 54 points. It is more time-efficient than the 30-2 and often preferred for glaucoma patients, focusing on areas where early damage occurs. 10-2 examines 10 degrees of the central visual field, with a denser grid (2-degree spacing) testing 68 points. It is used for detecting conditions affecting the central vision, such as macular or advanced glaucoma (178).

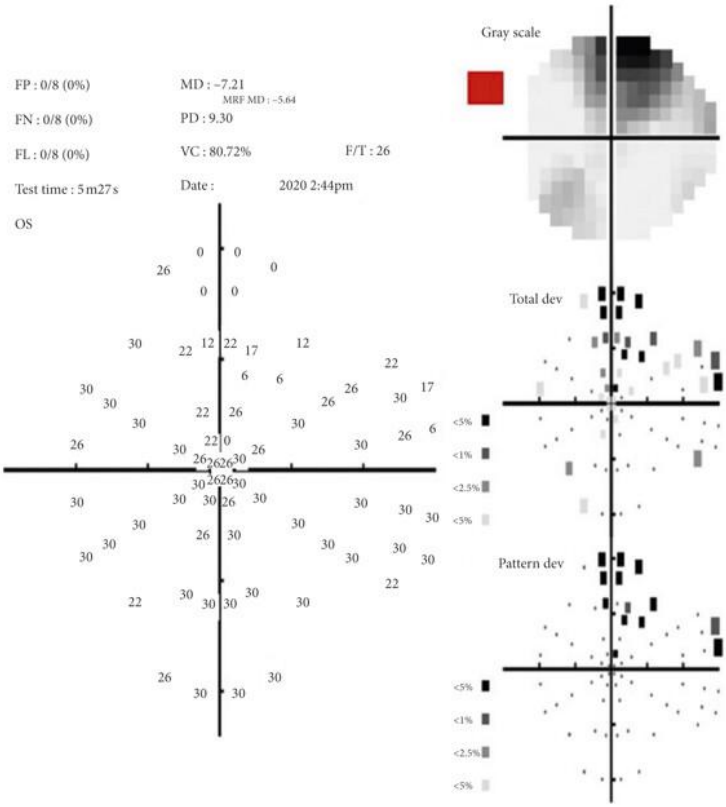



*Figure 2.3: <u>Representative Humphrey's visual field.</u> Image illustrating an example of an output of a Humphrey Visual field. The sensitivity is then transcribed to a greyscale image and visual field patterns are measured in decibels which is the limit of detection at certain points. False positives and false negative (labelled FP and FN) are used to determine the reliability and validity of the test. From (178).*

All visual field data was collected using a 30-2 setting, but where visual acuity was severely affected other settings such as 24-2 or 10-2 were utilised, and this was documented. The analysis of visual field data depends on looking at changes of sensitivity compared to a mean of known sensitivity in certain locations in the field, with -2Db corresponding to abnormality (179). Other data recorded included the distribution of the visual field loss, which could be a predictor of concomitant pathology (for example, a larger blindspot can point towards an increased in intraocular/intracranial pressure) (179).

### 2.3.3 Optical coherence tomography (OCT)

OCT data was obtained using the Zeiss 6000 Cirus machine in all cases. OCT is a non-invasive imaging technique that uses light waves to take cross-sectional pictures of the retina, allowing detailed visualisation of its layers to aid in diagnosing and monitoring retinal conditions like macular degeneration and glaucoma (180). It provides high-resolution images to help look at the structural integrity of the retina and optic nerve. Data was collected from initial and follow up OCT examinations performed on all patients. This included the retinal nerve fibre layer thickness in either eye in micrometers and the central macular thickness in either eye in micrometers. The abnormal value was when the value of the retinal nerve fibre layer and central macular thickness were more than normal for age according to the machine's standardised values. Macular oedema was assessed based on central retinal thickness; and verified by the ophthalmologist. Data for every visit was collected until latest follow up with data last checked on the 6$^{th}$ of August 2024.

### 2.3.4 Fluorescein angiography (FA) and indocyanine green analysis (ICGA)

FA and ICGA are imaging techniques used to visualise blood flow in the retina and choroid, respectively, often employed together in the California Protocol to diagnose retinal diseases like age-related macular degeneration and polypoidal choroidal vasculopathy. FA involves injecting fluorescein dye into a vein and capturing images as the dye travels through the retinal vasculature. It highlights abnormal blood vessels, leakage, and areas of ischemia in the retina (181).

ICGA uses indocyanine green dye, which is infrared-based, making it particularly effective for visualising the deeper choroidal vessels beneath the retina. It helps in detecting abnormalities in

conditions like choroidal neovascularisation and posterior choroidal vasculopathy (PCV) (182). Data was collected at any documented angiogram examination.

## 2.4 Radiological data

We also included data of imaging results if they are relevant to the diagnosis and treatment of uveitis. This was either through the central electronic medical record, or outpatient radiological information. This included sacroiliac X-ray, computer tomography (CT) or magnetic resonance imaging (MRI) looking for sacroiliitis; MRI of the brain to evaluate for concurrent neurological disease, and/or position emission tomography and CT chest abdomen pelvis to evaluate for concurrent systemic immunological or infiltrative disease or tumours.

## 2.5 Serum cytokine/chemokine profiling

Cytokine, chemokine and growth factor levels were measured in patient serum samples, age- and sex-matched healthy controls and MS controls. Samples were collected, spun for 10 min at 1300 g, the supernatant was aliquoted and frozen at -80ºC until analysis. We used the following Merck Millipore® MILLIPLEX® assays: Human Cytokine/Chemokine/Growth Factor Panel A (#HCYTA-60K-26-plex) to measure eotaxin (C-C motif chemokine ligand 11 (CCL11)), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage (GM)-CSF, GROα (C-X-C motif chemokine ligand-1 (CXCL1)), interferon (IFN) α2, IFN-γ, IL-1β, IL-1 receptor antagonist (IL-1RA), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 (p70), IL-13, IL-17A, IL-20, IFN-γ inducible protein 10 (IP-10, CXCL10), monocyte chemoattractant protein-1 (MCP-1, CCL2), monokine induced by gamma interferon (MIG, CXCL9), macrophage inflammatory protein (MIP)-1α (CCL3), MIP-1β (CCL4), tumour necrosis factor (TNF)-α and vascular endothelial

growth factor A (VEGF-A); and Human Cytokine/Chemokine/Growth Factor Panel B (#HCYTB-60K-9-plex) to measure IL21, interferon–inducible T cell alpha chemoattractant (I-TAC, CXCL11), MIP-3β (CCL19), stromal cell-derived factor 1 (SDF-1, CXCL12), thymus and activation-regulated chemokine (TARC, CCL17), B cell-attracting chemokine 1 (BCA-1, CXCL13), a proliferation-inducing ligand (APRIL), B-cell activating factor (BAFF) and IL-23, according to the manufacturer's instructions.

Briefly, the included standard samples and quality control samples as well as the magnetic beads were prepared, and patient serum samples were thawed and spun down at 10,000 g for 10 min. The standard sample dilution series, the quality control samples (both mixed with appropriate serum matrix) and the patient sera were incubated together with magnetic beads specific for the selected cytokines/chemokines/growth factors overnight (16-18 h, protected from light) at 2-8°C while shaking at 800 RPM. Subsequently, the plate was washed three times with wash buffer using a handheld magnet and shaking at 500 RPM, followed by incubation with detection antibodies for 1 h at room temperature while shaking at 500 RPM. Next, streptavidin-phycoerythrin was added and the plate was incubated for another 30 min while shaking. The plate was washed three times again, beads were resuspended for 5 min while shaking in 150 µl of 1x sheath fluid (xMAP® Sheath Concentrate PLUS, RUO; Millipore® #4050023), and the plate was read on a Luminex® 200™ (LX10011005401, Luminex Corporation, Austin, Texas, The United States) using xPonent® software (version 3.1.971.0, Luminex Corporation, Austin, Texas, The United States). When reading the plate right away was not possible, plate was stored at 4°C and agitated for 10 min while shaking at 500 RPM at room temperature right before measurement. Standard curve fitting was optimised using Belysa® software (version 1.0.19, Merck, Darmstadt, Germany).

## 2.6 Statistical analysis plan

Jamovi (Version 2.3.9, Jamovi), Microsoft Excel (version202402, Microsoft Office) ,SPSS (version 29.0.0.0, IBM), and R (version R-4.5. 1. tar. Gz) were used for statistical analysis. To analyse the differences between continuous and categorical variables, we employed the Kruskal-Wallis test, a non-parametric statistical method particularly suited for comparing multiple independent groups when the assumptions of normality or homogeneity of variance are not met. This test was chosen because our dataset included non-normally distributed continuous variables and categorical variables with more than two levels, making it an appropriate tool for detecting significant differences across groups. Descriptive statistics were first computed for clinical and historical data where quantifiable measures were available, providing a foundational understanding of the dataset. The primary objective of the analysis was to explore and identify potential differences in key variables based on aetiology.

Cytokine/chemokine multiplex data was analysed using Belysa® Immunoassay Curve Fitting Software (version 1.0.19, Merck) to calculate cytokine/chemokine concentrations, and the data was batch-corrected using 2 reference samples in each batch. R Studio (version 2025.05.0, Posit PBC; packages: tidyr, dplyr, factoextra, ggpubr, ggplot2, FactoMineR, mixOmics, broom, vegan, vegdist, pairwiseAdonis, pheatmap, tidyverse) and log2 transformed data without missing values was used for the principal component analysis (showing confidence ellipses) and Permutational Multivariate Analysis of Variance (Euclidean distance matrix, 999 permutations, p values were corrected for multiple comparisons using the Holm method). Shapiro-Wilk test was used to analyse data for normal distribution. The Spearman correlation heatmaps were calculated using pairwise.complete.obs and ward as the hierarchical clustering method.

Kruskal Wallis for general comparisons and post-hoc Dunn's for group comparisons with Bonferroni or Benjamini-Hochberg correction for multiple comparisons within disease groups were used, to ensure an exploratory approach to find cytokines/ chemokines of interest.

# Chapter 3 Clinical correlations

## 3.1 Cohort demographics

67 patients were prospectively recruited. The clinical characteristics and visual outcomes of patients presenting with acute uveitis were investigated. Our cohort included 36 (54%) females and 31 (46%) males.

Cohort level data was analysed with respect to patient demographics, clinical associations, and outcomes with statistical methodology outlined in Chapter 2. Briefly, Kruskal-Wallis testing was used for multilevel non-parametric analysis unless otherwise specified, given the multilevel nature of the independent variable and violation of assumption of normality of most data. We additionally performed pairwise comparison using the Dwass-Steel-Critchlow-Fligner method and a post-hoc Bonferroni correction to adjust for multiple comparisons.

The median duration of follow-up of patients in this cohort was 19 months (range 7-29 months). At the time of latest follow-up, 29/67 (43%) patients were classified as being in the immune-mediated group, 17/67 (25.4%) in the infectious group, and 21/67 (31%) in the idiopathic group (Figure 3.1). The gender distribution between aetiological groups was not significantly different (immune 18/29 female, 62%; infectious 6/17 female, 35%; idiopathic 12/21 female, 57%; p=0.203).

The diagnoses were mainly made by clinical assessment and directed investigations. In the immune-mediated group, patients with HLA-B27 related disorders were diagnosed based on a

positive HLA-B27 test in association with additional systemic manifestations such as ankylosing spondylitis or Crohn's disease. Patients with sarcoidosis were based on an associated clinical presentation in combination with an elevated angiotensin converting enzyme levels and/or histopathological confirmation. Patients with VKH were diagnosed based on their clinical presentation in conjunction with their OCT, fluorescein, and indocyanine findings. Vaccine related patients were diagnosed by multidisciplinary expert consensus Birdshot uveitis was diagnosed based on the birdshot appearance on dilated eye exam and with a confirmatory HLA-A 29 test. A patient deemed to have drug induced uveitis was on adalimumab for their psoriatic arthritis when they developed uveitis, and who improved with oral prednisolone and stopping the adalimumab. Their diagnosis was made based on the correlation of the symptoms to the cessation of the medication.

In the infectious group, patients diagnosed with uveitis due to syphilis, tuberculosis, toxoplasmosis, HSV and Bartonella had their diagnosis confirmed with a typical clinical presentation confirmed with relevant serology.

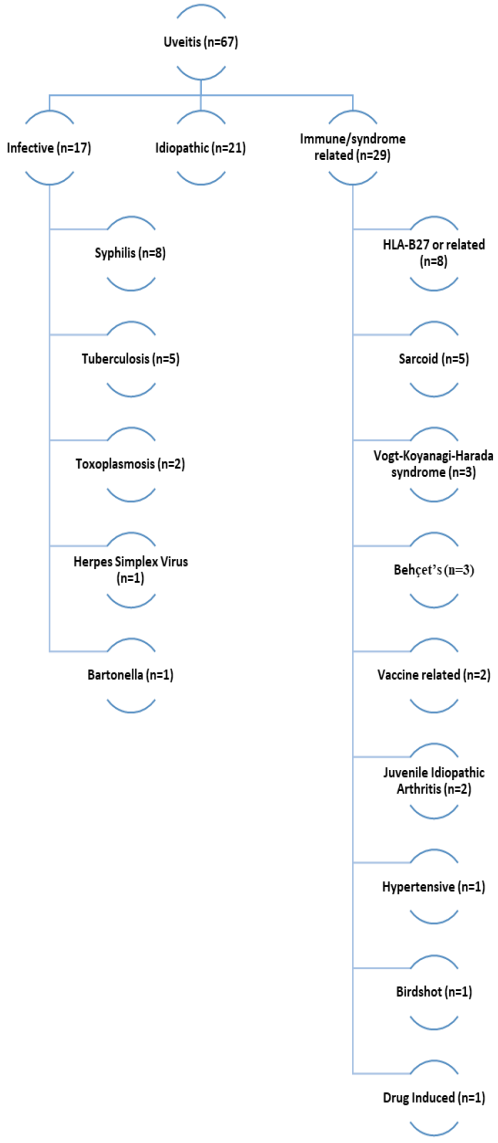



*Figure 3.1 Distribution of cause of each measured uveitis episode by aetiology. This diagnosis was based on consensus of uveitis specialists in tertiary clinics where the patients were recruited*

The ethnic distribution of participants (n=67) is illustrated in Figure 3.2, with Northern European individuals representing the largest demographic group, accounting for 30 participants (45%). This was followed by South Asian (15%) and Southeast Asian (12%) participants, comprising 10 and eight individuals, respectively. Smaller proportions were observed among participants of East Asian or Middle Eastern descent (6%), South European (7%), and Continental Asian (4%). Participants of North African and Continental European descent each accounted for 4% (n = 3),

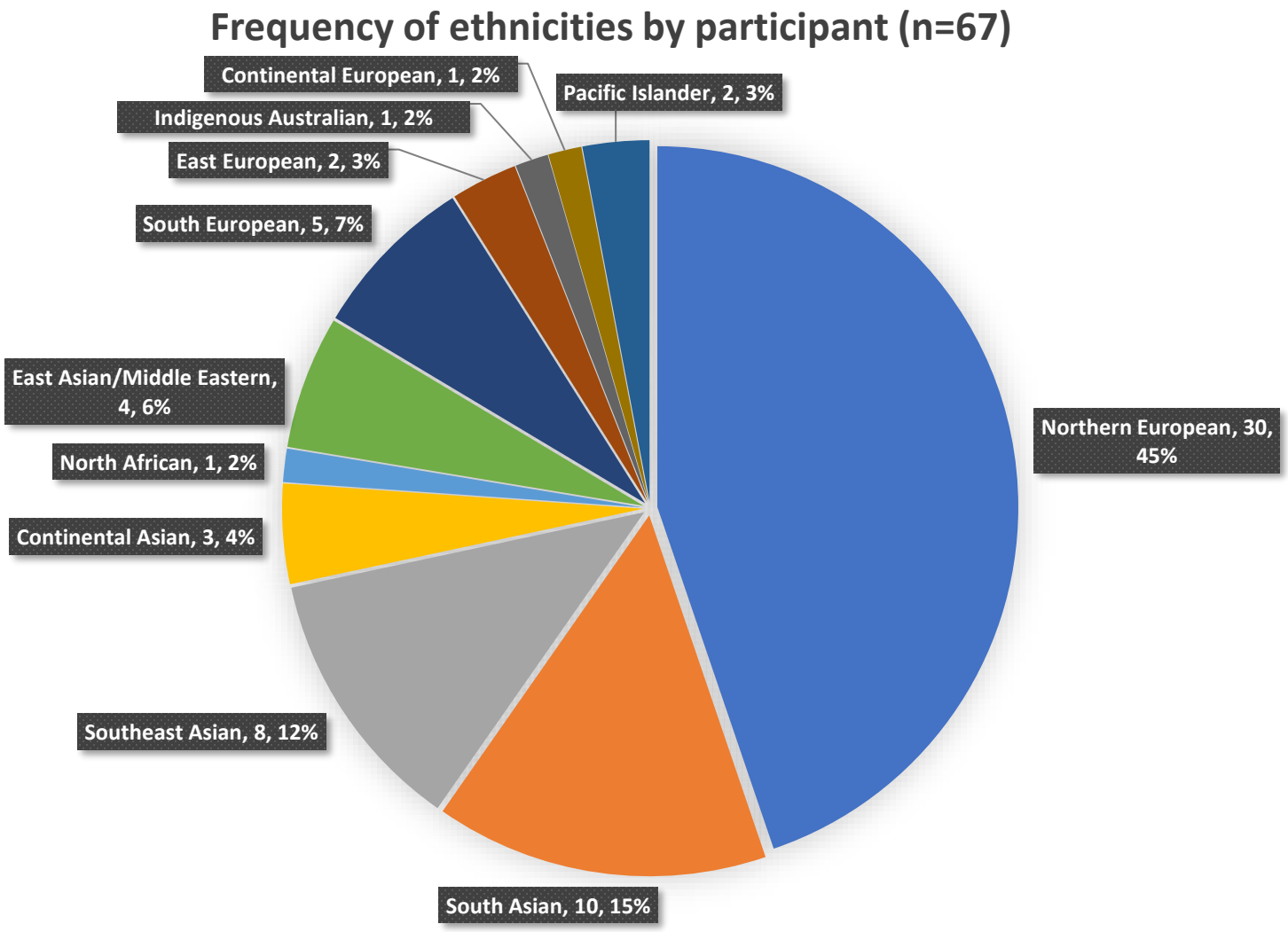



*Figure 3.2 Ethnic distribution of cohort*

while East European, Indigenous Australian, and Pacific Islander ethnicities were represented by one participant each (2%).

Patient recruitment took place between 2021 to 2023, mostly during the Covid pandemic. Due to reports of vaccine triggered episodes of uveitis, we documented proximity of vaccination to onset of acute uveitis in this cohort of patients. Among the 67 patients, 13 individuals (19%) had received a COVID-19 vaccine (either AstraZeneca or Pfizer) within one month of uveitis onset. 18 patients (27%) had received their vaccination more than one month prior to presentation (range: 1 month to 9 months with one patient refusing to get a COVID-19 vaccination). Two patients (3.0%) were unvaccinated, while vaccination status was unavailable for 11 individuals (16%). Among those with a known vaccination timepoint (n=31), the odds of presenting with uveitis within one month of COVID-19 vaccination were 0.72 times the odds of developing uveitis more than one month after vaccination.

## 3.2 Clinical data

Clinical data findings are summarised in Table 3.1. The median age at disease onset was similar across all three uveitis categories, 38 years (range: 4–59) in the immune group, 36.5 years (range: 17–59) in the infectious group, and 38 years (range: 4–77) in the idiopathic group (p=0.954). Similarly, the median age of patients at the time of the current episode of acute uveitis prompting recruitment to this study also showed no significant differences between aetiological groups.

Patients were recruited during an acute presentation of uveitis – either at first presentation of uveitis, or during a relapse. Patients with an infectious aetiology were mainly recruited at first presentation of uveitis (14/17, 82%), compared to only 11/29 (38%) of immune-mediated patients and 10/21 (48%) of patients with an idiopathic aetiology (p=0.014), who were mostly recruited during a relapse.

A relapsing disease course was more frequently identified in the immune-mediated group (20/29 patients, 69%), compared to the infectious group (3/17, 18%) and idiopathic group (12/21, 57%); p=0.024). This was mostly driven by relapses in the immune group compared to the infectious group (p=0.004). Patients with infectious uveitis most frequently had a monophasic course, with this identified in 14/17 (82%) of the patients. The median number of relapses in the immune-mediated group was 24 relapses (interquartile range (IQR 15-30)) compared to a median of 8 relapses in the infectious group (IQR 3-13), and 18 in the idiopathic group (IQR 7-29; p=0.003). Pairwise comparisons of these groups revealed this was primarily driven by a reduced number of relapses in the infectious group compared to the immune-mediated group (p=0.0024).

There were no statistically significant differences between aetiological groups regarding laterality of uveitis, uveitis classification (anterior, intermediate, posterior or panuveitis), or the presence of optic disc swelling or optic neuritis.

The immune-mediated uveitis patients more frequently had a coexisting autoimmune systemic disorder (18/27, 67%) compared to the other groups (p=0.00008). This was higher compared to both the infectious (29%, p=0.045), and the idiopathic group (5%, p=0.00007). The associated

systemic disorders in the immune-mediated group included HLA-B27 related disease (n=13), juvenile rheumatoid arthritis (n=2), Behcet's disease (n=2), idiopathic arthritis, sarcoidosis (n=2), VKH (n=2). However, the presence of active features of systemic autoimmunity such as a rash, joint pain and swelling, mouth ulcers, serositis, fevers and lymphadenopathy, headaches, diarrhoea, at the time of clinical evaluation at recruitment were not significant between the three groups (41% in immune, 24% in idiopathic and 19% in infectious, p=0.216).

A similar proportion of immune-mediated (10/27, 37%) and idiopathic patients (7/20, 35%) reported a family history of autoimmunity in first degree relatives, whereas no patient from infectious group did (p = 0.016).

Self-reported eye pain was further characterised, including pain at nadir, pain with eye movement, pain with sleep, and pain at latest follow-up. There were no significant differences between aetiological groups. Median pain scores at nadir ranged from 1 to 3 out of 10, and were reduced to 0 in all groups at follow-up, suggesting clinical resolution or effective management of symptoms in all three groups of patients..

Among patients with an immune-mediated aetiology (n = 29), the duration of follow-up ranged from 4 to 37 months, with a median of 24 months. There were no missing values in this group. In the infectious group (n = 16), follow-up duration was shorter, ranging from 4 to 33 months, with a median of 8.5 months; one case had missing follow-up data and was lost to follow up. For the idiopathic group (n = 20), follow-up ranged from 3 to 36 months, with a median of 18.5 months, with one missing value recorded due to loss to follow up. Kruskal-Wallis of all three groups

demonstrates a significant result of p=0.002. This difference was largely driven by the longer follow-up duration of the immune-mediated group compared to the infectious group (p=0.028).

Autoimmune markers were tested in 50/67 patients (74.62%) of patients as part of their work up. Out of those patients 12/50 (24%) had a weakly positive ANA 1:80, which is usually not felt to be a positive investigation. Eight patients ( 16%) had strongly positive ANAs. Of these patients, four were idiopathic patients, three were infectious patients and one was immune patient. Two patients had positive ANCA ENA positivity, both of whom are idiopathic patients. This was thus, not used as a way to determine aetiology of diagnosis despite being part of the “screening” uveitis bloods.

**Table 3.1 Clinical features of uveitis patients with immune-mediated, infectious and idiopathic aetiologies**

| | | Total patients n=67 | Immune n=29 | Infectious n=17 | Idiopathic n=21 | *p value* | *Pairwise Comparison p value* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *immune-infectious* | *immune-idiopathic* | *infectious-idiopathic* |
| Gender female | | 36/67 (54%) | 18/29 (62%) | 6/17 (35%) | 12/21 (57%) | 0.203 | 0.192 | 0.936 | 0.382 |
| Median age at onset of uveitis | | 38 (4-77) | 38 (4-59) | 36.5 (17-59) | 38 (4-77) | 0.954 | 0.967 | 0.994 | 0.954 |
| Median age at onset of current uveitis episode | | 43 (16-78) | 44 (17-72) | 38 (18-45) | 43 (16-78) | 0.680 | 0.876 | 0.681 | 0.923 |
| Days from onset to blood sample collection (median, range) | | 33.5 (1-154) | 39 (1-154) | 31 (5-85) | 27(4-129) | 0.837 | 0.793 | 0.975 | 0.968 |
| Clinical course (%) | Relapsing | 35/67 (52%) | 20/29 (69%) | 3/17 (18%) | 12/21 (57%) | ***0.024**** | **0.004**** | *0.672* | 0.053 |
| | Monophasic | 32/67 (48%) | 9/29 (31%) | 14/17 (82%) | 9/21 (43%) | | | | |
| Initial episode (%) | | 35/67 (52%) | 11/29 (38%) | 14/17 (82%) | 10/21 (48%) | ***0.014**** | **0.011*** | *0.776* | 0.075 |
| Number of relapses (median, IQR) | | 18 (6.5-28.5) | 24 (15-30) | 8 (3-13) | 18 (7-29) | ***0.003**** | **0.0024*** | 0.33771 | 0.084 |
| Laterality: bilateral (%) | | 29/66 (44%) | 12/29 (41%) | 7/29 (24%) | 10/29 (34%) | 0.125 | 0.795 | 0.483 | 0.876 |
| Uveitis location (%) | anterior | 25/67 | 12/29 (41%) | 4/17 (24%) | 9/21 (43%) | 0.180 | 0.338 | 0.821 | 0.182 |
| | intermediate | 6/67 (9%) | 1/29 (3%) | 2/17 (12%) | 3/21(14%) | | | | |
| | posterior | 15/67 (22%) | 5/29 (17%) | 7/17 (41%) | 3/21 (14%) | | | | |
| | panuveitis | 15/67 (22%) | 7/29 (24%) | 3/17 (18%) | 5/21 (24%) | | | | |
| | other (combination) | 6/67 (9%) | 4/29 (14%) | 1/17 (6%) | 1/21 (5%) | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Presence of optic disc swelling (%) | 19/66 (29%) | 7/28 (25%) | 6/17 (35%) | 6/21 (29%) | 0.758 | 0.770 | 0.848 | 0.986 |
| Presence of optic neuritis (%) | 16/65 (25%) | 7/28 (25%) | 5/17 (29%) | 4/20 (20%) | 0.769 | 0.945 | 0.891 | 0.745 |
| Coexisting systemic autoimmunity/disease(%) | 24/64 (38%) | 18/27 (67%) | 5/17 (29%) | 1/20 (5%) | ***0.00008**** | **0.045*** | **0.00007**** | 0.117 |
| Presence of active features consistent with systemic autoimmunity (%) | 20/66 (30%) | 12/29 (41%) | 3/16 (19%) | 5/21 (24%) | 0.216 | 0.280 | 0.406 | 0.929 |
| Family history of autoimmune disease (%) | 17/64 (27%) | 10/27 (37%) | 0/17 (0%) | 7/20 (35%) | ***0.016**** | **0.013*** | 0.989 | **0.021*** |
| Pain at nadir (0-10) (median, range) | 2 (0-10) | 2 (0-8) | 1 (0-9) | 3 (0-10) | 0.392 | 0.703 | 0.629 | 0.442 |
| Pain with sleep present (%) | 20/64 (31%) | 6/27 (22%) | 3/17 (18%) | 4/20 (20%) | 0.935 | 0.982 | 0.930 | 0.982 |
| Pain with eye movement (%) | 20/64 (31%) | 9/27 (33%) | 4/17 (24%) | 7/20 (35%) | 0.769 | 0.992 | 0.772 | 0.734 |
| Pain at follow up (0-10) (median, range) | 0 (0-6) | 0 (0-3) | 0 (0-1) | 0 (0-6) | 0.131 | 0.236 | 0.311 | 0.987 |
| Duration of follow up in months (median, range) | 19 (7-29) | 24 (4-37) | 8.5 (4-33) | 18.5 (3-36) | ***0.002**** | **0.028*** | 0.675 | 0.106 |
| Meds associated with uveitis (%) | 18/67 (27%) | 11/29 (38%) | 5/17 (29%) | 2/21% (10%) | 0.082 | 0.831 | 0.065 | 0.267 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Ocular/ophthalmic complications | 34/67 (50.7%) | 13/27(48.2%) | 8/17 (47%) | 13/21(62%) | 0.563 | 1 | 0.511 | 0.577 |

This table shows clinical data across the three (immune, idiopathic and infectious) groups. The Kruskal-Wallis test was used for group comparisons and the Dwass-Steel-Critchlow-Fligner test was used for pairwise comparisons. Significant p values are indicated by asterisks: *: p⩽0.05, **: p⩽0.01, ****p⩽0.0001. The p-values shown include post-hoc Bonferroni correction to adjust for multiple comparisons.

## 3.3 Ancillary investigations

A fluorescein angiogram was performed when it was deemed appropriate by a clinician and patients gave consent. 27/67 (40%) patients had a fluorescein angiogram investigation in our cohort. Out of these patients 11/27 (41%) had a normal or unremarkable angiogram. 3/27 number that had a pathological feature presented with two concomitant abnormalities. Observed clinical observations included: vasculitis in four cases, vascular leak in three cases, neovascularisation in two cases, retinitis in two cases and choroiditis in two cases. Hyperautofluorescene, keratitic papules, macular star, serpiginous vessel, capillary occlusion, and a raised disc was noted in one patient each. Nine patients proceeded to have an indocyanine green angiography with three patients confirmed to have a vascular leak and, all of whom had panuveitis.

## 3.4 Therapeutic approaches

All patients in this cohort received at least one modality of treatment. 43/67 (64%) received topical corticosteroids. 42/67 (63%) patients had oral corticosteroids for more than three days. This was likely related to corticosteroids being prescribed by the emergency department while patients awaited follow up in ophthalmology clinics. 4/67 (6%) patients received intravenous corticosteroids. In the idiopathic group and immune-mediated group, all bar one idiopathic patient received topical steroids. 15/27 (56%) patients in the immune-mediated group were on steroid-sparing therapy. Five were on methotrexate (33%), six received a TNF-α inhibitor (40%), two were on azathioprine (13%) and two were on mycophenolate 1. 3/15 (20%) patients were on

methotrexate and prednisolone at the same time for a prolonged period. 1/15 (6.67%) patients was on both TNF-α inhibitor and methotrexate with attempts to wean the methotrexate at the time of last review.

In the immune-mediated group, all patients received immunotherapy. This included patients oral steroid sparing immunosuppression including azathioprine (two patients) or methotrexate (seven patients), either with corticosteroids as an adjunct, or due to steroid side effects. 11/29 (38%) patients received adalimumab to treat ankylosing spondolyitis. 10/29 patients were treated with mycophenolate, nine with methotrexate, three with intravenous immunoglobulin, and two with tacrolimus to treat uveitis associated with VKH. One patient with a diagnosis of juvenile rheumatoid arthritis with multi-systemic manifestations including uveitis, received a combination of rituximab, certolizumab, azathioprine and leflunomide.

25/67 (37%) of patients received antimicrobial therapy. This included all 17 patients in the infectious group, seven in the immune-mediated group, and one patient in the idiopathic group. 5/67 (7.5%) patients were given IV benzyl penicillin, four in the infectious group for treatment of syphilis, and one patient in immune-mediated group for empirical management of unexplained fevers. 11/67 (16%) patients received trimethoprim sulfamethoxazole for *pneumocystis jirovecii* prophylaxis, while on high doses of prednisolone. Nine of these patients were in the immune-mediated group and two were in the infectious group. 6/67 (9%) patients were treated with valacyclovir (three immune-mediated, three infectious patients) and 3/67 (4.5%) patients received intraocular antivirals, for HSV uveitis (n=2) and suspected HSV uveitis (n=1). 2/67 (3%) patients in the infectious group received doxycycline for the treatment of bartonella and as second line

treatment for toxoplasmosis (n=1 each). 4/67 (6%) patients received combination therapy for tuberculous uveitis (rifampicin, isoniazid, pyrazinamide, ethambutol and vitamin B6 supplementation), with an additional patient presenting with tuberculosis uveitis treated with topical steroids only. Some treatments were only used by a single patient as detailed: entecavir (immune-mediated patient due to hepatitis B core positivity while on concurrent steroids), ciprofloxacin (2nd line anti tubercular therapy), augmentin (indication unclear in immune-mediated patient), oral clindamycin (empiric therapy in an idiopathic patient with fevers) and another patient under the infectious group who received clindamycin for toxoplasma treatment, idiopathic patient, cephazolin (infectious patient with bartonella uveitis and an allergic reaction to ceftriaxone).

## 3.5 Visual function and outcomes

Visual outcomes were assessed in 95 affected eyes in the uveitis cohort (39 eyes immune-mediated group, 24 eyes infectious group, 32 eyes idiopathic group, Table 3.2). A Kruskal Wallis test was used to analyse differences between the three groups, followed by pairwise comparisons using the Dwass-Steel-Kritchkow-Fligner test. Bonferroni corrections were applied to adjust for multiple comparisons.

**Table 3.2 Visual outcomes in affected eyes**

| | | Total N eyes=95 | Immune n=39 | Infectious n=24 | Idiopathic n=32 | p-value | *pairwise comparisons p value* | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | *immune-infectious* | *immune-idiopathic* | *infectious-idiopathic* |
| VA nadir logmar (median:IQR) | | 0.34 [0.09-0.74] | 0.3 [0.01-0.53] | 0.41 [0.17-0.79] | 0.37 [0.12-0.77] | 0.37 | 0.331 | 0.728 | 0.835 |
| VA latest follow up logmar (median:IQR) | | 0.14 [0-0.26] | 0 [0-0.19] | 0.16 [0.02-0.33] | 0.21 [0-0.31] | 0.053 | 0.086 | 0.117 | 0.999 |
| RNFL nadir (µm) (median:IQR) | | 106 [94-131] | 106 [95-132] | 105.5 [94.5-120.8] | 107 [94.8-122.8] | 0.99 | 0.999 | 0.991 | 0.993 |
| RNFL latest follow up (µm) (median:IQR) | | 101 [82-111] | 101 [84-111] | 100 [84-110.5] | 101 [74.8-112.5] | 0.91 | 0.955 | 0.912 | 0.998 |
| macular thickness layer at nadir (µm) (median:IQR) | | 269 [247-288] | 270 [259.5-292.5] | 250 [222.5-277] | 269 [250-303.3] | **0.037*** | **0.030*** | 0.925 | 0.126 |
| Macular thickness layer at latest follow-up (µm) (median:IQR) | | 261.5 [237-277] | 263 [243-276.5] | 253 [216.5-271] | 264 [239.5-278] | 0.27 | 0.304 | 0.999 | 0.324 |
| visual fields (dB) (median:IQR) | | -4.8 [-13.3- -1.1] | -2.3 [-7.3- -0.67] | -8.8 [-19.9- -3.8] | -4.5 [-13.1- -1.9] | 0.34 | 0.364 | 0.590 | 0.791 |
| field loss type (%) | normal | 12/39 (31%0 | 5/14 (35.7%) | 4/13 (30.1%) | 3/12 (25%) | 0.56 | 0.990 | 0.643 | 0.990 |
| | centrocaecal | 5/39 (13%) | 1/14 (7.1%) | 1/13 (7.7%) | 3/12 (25%) | | | | |
| | peripheral | 2/39 (5%) | 0/14 (0%) | 1/13 (7.7%) | 1/12 (8.3%) | | | | |
| | diffuse | 7/39 (18%) | 1/14 (7.1%) | 2/13 (15.4%) | 4/12 (33%) | | | | |
| | altitudinal | 6/39 (15%) | 3/14 (21.4%) | 3/13 (23.1%) | 0/12 (0%) | | | | |
| | increased blind spot | 6/39 (15%) | 3/14 (21.4%) | 2/13 (15.4%) | 1/12 (8.3%) | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| other | 1/39 (3%) | 1/14 (7.1%) | 0/13(0%) | 0/12 (0%) | | | | |
| macular oedema at nadir | 31/92 (33.7%) | 9/39 (23.1%) | 8/23 (25%) | 14/30 (46.7%) | 0.12 | 0.583 | 0.583 | 0.665 |
| macular oedema at latest follow-up | 10/92 (10.9%) | 3/39 (7.7%) | 2/23 (8.7%) | 5/30 (16.6%) | 0.46 | 0.989 | 0.989 | 0.677 |
| Intraocular pressure at nadir (median:IQR) | 14 [11.25-16.75] | 14 [11-17] | 14[11-18] | 14 [12-15] | 0.84 | 0.987 | 0.869 | 0.876 |
| Intraocular pressure at latest follow up(median:IQR) | 13.5 [11-15] | 13[10-15.5] | 14[11-15.25] | 14 [12-14.5] | 0.84 | 0.808 | 0.951 | 0.976 |

This table shows the clinical features of affected eyes grouped by immune, infectious and idiopathic uveitis aetiologies. Group were compared using the Kruskal-Wallis test and pairwise comparison was done using the Dwass-Steel-Critchlow-Fligner correction. The p-values shown include post-hoc Bonferroni corrections to adjust for multiple comparisons. Significant p values are indicated by asterisks: *: $p \leqslant 0.05$, **: $p \leqslant 0.01$, ****$p \leqslant 0.0001$. OCT:- Ocular coherence tomography, RNFL: Retinal nerve fibre layer. VA: Visual acuity,

Visual acuity was measured using the LogMAR transformation as previously validated (183) and as described in Chapter 2. Visual acuity (VA) at nadir did not show any significant differences across the three groups (p=0.37, median (IQR) – immune-mediated (0.3, 0.01-0.53), infectious 0.41, 0.17-0.79) and idiopathic (0.37, 0.12-0.77). However, a trend towards better outcomes in the immune-mediated group compared to the infectious group at latest follow-up (p=0.086) was observed (immune-mediated 0 (0-0.19) versus infectious median logMAR VA 0.16 (0.02-0.33), suggesting potentially more visual recovery in immune uveitis.

The retinal nerve fibre layer (RNFL) thickness at nadir and at follow up was not significant between the three groups, either at nadir or at latest follow-up. Macular oedema at nadir was observed in more than one third of patients with uveitis (31/92 eyes, 34%) compared to macular oedema at follow-up (10/92 eyes, 11%). However, the groups did not have any statistically significant differences in rates of macular oedema at nadir nor at follow up (p= 0.12 and p=0.46 respectively). The macular layer thickness at nadir was different in the groups tested (p=0.037). The difference in pairwise comparisons was seen primarily in the immune-mediated group (immune-mediated 270 (259.5-292.5), which was significantly higher at nadir than in the infectious group (250, 222.5-277, p=0.030). No significant differences in macular layer thickness were found at last follow-up.

There were no significant differences in overall visual field measurements between aetiological categories (p=0.34). Furthermore, there was no significant difference in the type of visual field

defect between aetiologies. The presence of macular oedema at nadir and at latest follow up was not significantly different between the groups (p=0.12 and p=0.46 respectively).

Intraocular pressure was not different at nadir or at latest follow up in all 3 groups and was not different before the introcular pressure group as a whole at nadir and at follow up.

Approximately half of the patients (34/67=50.74%) developed a concomitant ophthalmic diagnosis or complication, either as a complication of the disease of which they are diagnosed with, a complication of the treatment of the current uveitis episode, or because of previous uveitis episodes or treatments. In the immune-mediated group, 13/27 (48.15%) had a concurrent ophthalmic complication. Three patients (11.11%) developed cataracts as a result of the condition, and one required cataract surgery. Two (7.47%) patients developed glaucoma, and two needed a vitrectomy. Two patients developed a lacrimal gland infection and one patient (3.70%) required a tuberectromy. One patient developed cystoid macular oedema, one had a tear duct blockage and one developed corneal clouding. In the infectious group, 8/17 (47%) of patients had ophthalmologic complications. Three (17.65%) patients had cataracts, two (11.76%) had glaucoma, one (5.88%) developed optic atrophy, and one developed retinal inferior right lateral ischemia. In the idiopathic group, 13/21 (62%) patients developed complications. 5/21 (23.81%) developed cataracts, of whom three required surgery. Two (9.52%) developed optic atrophy. One (4.76%) patient needed a vitrectomy and keratectomy. One patient developed synchioloysis and another patient developed iris bombe. One patient developed glaucoma and that required trabeculectomy.

# Chapter 4 Cytokine analysis in acute uveitis

## 4.1 Cytokine analysis.

To characterise immune, infectious and idiopathic uveitis patients in more detail, we next sought to measure and analyse the concentration of 35 cytokines and chemokines in the serum of our patients with acute uveitis. Merck Milliplex® multiplex assays were use, which enables testing multiple proteins in one sample simultaneously. The following cytokines and chemokines were measured in the serum of 65 uveitis patients and 30 age-matched healthy controls (HC): APRIL (A Proliferation-Inducing Ligand), BAFF (B-cell Activating Factor), BCA-1 (B-cell Attracting Chemokine-1 or CXCL13), Eotaxin, G-CSF (Granulocyte Colony-Stimulating Factor), GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), GRO-α (Growth-Regulated Oncogene-alpha or CXCL1),IFN-α2 ,IFN-γ (Interferon-gamma), I-TAC (Interferon-Inducible T-cell Alpha Chemoattractant or CXCL11), IL-1 β (IL-1β), IL-1ra (IL-1 Receptor Antagonist), IL-2 , IL-4 , IL-5 , IL-6 , IL-8 (or CXCL8), IL-10 , IL-12p70 (IL-12 p70 heterodimer), IL-13, IL-15 , IL-17α , IL-21, IL-22 , IL-23 , IP-10 (Interferon Gamma-Induced Protein 10 or CXCL10), MCP-1 (Monocyte Chemoattractant Protein-1 or CCL2), MIG (Monokine Induced by Gamma Interferon or CXCL9), MIP-1α (Macrophage Inflammatory Protein-1α or CCL3), MIP-1β (Macrophage Inflammatory Protein-1β or CCL4), MIP-3β (Macrophage Inflammatory Protein-3β or CCL19), SDF-1 (Stromal Cell-Derived Factor-1 or CXCL12), TARC (Thymus and Activation-Regulated Chemokine or CCL17), TNF-α (Tumor Necrosis Factor-alpha) and VEGF-α (Vascular Endothelial Growth Factor-alpha . These cytokines were compared to sera of healthy controls and to each other in statistical analysis.

## 4.2 Healthy control with three aetiological groups comparisons

Data on cytokines is summarized in the table 4.1.

**Table 4.1 Comparison of 35 cytokine levels across healthy controls (HC), immune, infectious, idiopathic groups then a second comparsions without healthy controls**

| *Cytokines n patients=95 n cytokines=35* | *HC (pg/ml) median [IQR] n=30* | *Immune in pg/ml [Median IQR] n=25* | *Infectious in pg/ml [Median IQR] n=18* | *Idiopathic in pg/ml [Median IQR] n=21* | *P Value for general test of 4 HC vs group* | *Pairwise comparisons p value (HC vs group)* | | | *P value for General test of disease group vs disease groups* | *Pairwise comparisons p value (disease group vs disease group)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *HC Immune* | *HC infectious* | *HC Idiopathic* | | *Immune infectious* | *Immune Idiopathic* | *Infectious idiopathic* |
| APRIL | 3571 [2664-4257] | 3400 [2712-3815] | 3512 [3020-4862] | 3562 [2948-4421] | 0.574 | 1 | 1 | 1 | 0.333 | 0.460202 | 0.460202 | 0.696213 |
| BAFF | 1740 [1579-2090] | 1799 [1239-2275] | 1508 [1339-2373] | 1939 [1323-2679] | 0.74 | 1 | 1 | 1 | 0.624 | 0.734908 | 0.725273 | 0.725273 |
| BCA-1 | 40.4 [33.41-58.82] | 62.66 [45.61-80.31] | 82.61 [60.65-176.7] | 77.64 [49.89-120] | **0.000073**** | **0.048614*** | **0.000060**** | **0.002258**** | 0.0846 | 0.082498 | 0.295180 | 0.295180 |
| Eotaxin | 213.1 [139.6-246.2] | 223 [153.1-282.9] | 228.3 [134.7-262.6] | 220.2 [126.7-303.2] | 0.925 | 1 | 1 | 1 | 0.826 | 0.869101 | 0.869101 | 0.869101 |
| G-CSF | 63.47 [49.1-107.1] | 58.46 [40.76-92.44] | 48.18 [27.98-78.33] | 86.3 [24.18-86.3] | 0.155 | 1 | 0.294327 | 1 | 0.0917 | 0.305941 | 0.277034 | 0.089277 |
| GM-CSF | 7.948 [6.36-23.72] | 11.08 [7.631-103] | 7.631 [1.55-33.11] | 11.45 [7.084-194.9] | 0.157 | 0.657963 | 0.984003 | 0.861039 | 0.114 | 0.121109 | 0.990474 | 0.121109 |

| *Cytokines* ***n patients=95 n cytokines= 35*** | *HC (pg/ml) median [IQR]* ***n=30*** | *Immune in pg/ml [Median IQR] n=25* | *Infectious in pg/ml [Median IQR] n=18* | *Idiopathic in pg/ml [Median IQR] n=21* | *P Value for general test of 4 HC vs group* | *Pairwise comparisons p.value (HC vs group)* | | | *P value for General test of disease group vs disease groups* | *Pairwise comparisons p value (disease group vs disease group)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *HC Immune* | *HC infectious* | *HC Idiopathic* | | *Immune infectious* | *Immune Idiopathic* | *Infectious idiopathic* |
| GRO-α | 48.75 [31.96-79.32] | 44.7 [35.35-58.13] | 30.91 [23.68-38.17] | 55.51 [30.79-65.73] | **0.0119** | 1 | **0.007117*** | 1 | **0.0136** | **0.018767*** | 0.742858 | **0.018767*** |
| IFN-α2 | 46.46 [20.04-60.14] | 27.54 [18.66-47.59] | 23.1 [11.49-39.41] | 27.27 [5.827-38.91] | 0.0603 | 1 | 0.051872 | 0.139116 | 0.238 | 0.294213 | 0.294213 | 0.839659 |
| IFN-γ | 71.89 [34.43-149] | 87.58 [43.56-206.2] | 145 [79.48-169.1] | 153.1 [25.94-302.8] | 0.195 | 0.637694 | 0.189407 | 0.263495 | 0.781 | 0.847237 | 0.847237 | 0.991881 |
| IL-10 | 10.31 [5.807-28.9] | 11.68 [5.912-36.45] | 10.87 [6.958-20.19] | 14.32 [8.905-37.24] | 0.642 | 1 | 1 | 0.782692 | 0.526 | 0.537813 | 0.537813 | 0.537813 |
| IL-12p70 | 5.623 [2.833-8.241] | 4.269 [2.245-15.35] | 3.969 [2.245-15.66] | 5.935 [3.352-27.13] | 0.67 | 1 | 1 | 1 | 0.438 | 0.827632 | 0.425128 | 0.425128 |
| IL-13 | 81.88 [57.05-136.5] | 111.9 [70.07-234.2] | 72 [55.08-188.2] | 90.92 [39.85-309.5] | 0.31 | 0.188778 | 1 | 1 | 0.486 | 0.569473 | 0.569473 | 0.824248 |
| IL-15 | 13.9 [10.52-19.87] | 13.6 [10.32-19.3] | 8.858 [7.048-13.83] | 13.37 [6.714-21.34] | 0.174 | 1 | 0.12138 | 1 | 0.188 | 0.212442 | 0.612017 | 0.354046 |

| *Cytokines* ***n patients=95 n cytokines= 35*** | *HC (pg/ml) median [IQR]* ***n=30*** | *Immune in pg/ml [Median IQR] n=25* | *Infectious in pg/ml [Median IQR] n=18* | *Idiopathic in pg/ml [Median IQR] n=21* | *P Value for general test of 4 HC vs group* | *Pairwise comparisons p.value (HC vs group)* | | | *P value for General test of disease group vs disease groups* | *Pairwise comparisons p value (disease group vs disease group)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *HC Immune* | *HC infectious* | *HC Idiopathic* | | *Immune infectious* | *Immune Idiopathic* | *Infectious idiopathic* |
| IL-17α | 32.93 [14.19-42.57] | 25.95 [9.689-55.17] | 5.559 [2.444-10.78] | 18.31 [10.16-59.27] | **0.00412*** ** | 1 | **0.001745** ** | 1 | **0.00775** ** | **0.009755** ** | 0.852891 | **0.016723*** |
| IL-1β | 10.13 [5.325-21.25] | 10.22 [3.726-20.25] | 3.726 [1.115-7.054] | 8.141 [0.4054-29.49] | 0.0922 | 1 | 0.098191 | 0.812628 | 0.101 | 0.106444 | 0.339845 | 0.386772 |
| IL-1Rα | 6.201 [4.77-9.46] | 7.753 [5.312-13.81] | 10.21 [3.184-15.7] | 7.004 [3.282-24.81] | 0.936 | 1 | 1 | 1 | 0.956 | 0.906014 | 0.906014 | 0.906014 |
| IL-2 | 1.6 [0.737-2.923] | 2.087 [0.7383-6.35] | 1.066[0.3615-2.095] | 0.9059[0.3532-3.553] | 0.258 | 1 | 1 | 1 | 0.193 | 0.22414 | 0.22414 | 0.818879 |
| IL-21 | 1.69 [0.69-7.12] | 4.275 [0.9925-23.7] | 1.84 [0.69-5.225] | 2.955 [0.69-21.42] | 0.972 | 0.287886 | 1 | 1 | 0.105 | 0.101374 | 0.449249 | 0.286922 |
| IL-22 | 154 [89-223.6] | 207.3 [125.7-564.6] | 169.8 [146.6-314.4] | 219.8 [180.6-407.4] | 0.0869 | 0.227963 | 0.911165 | 0.076882 | 0.555 | 0.629203 | 0.629203 | 0.629203 |
| IL-23 | 37.34 [4.99-82.22] | 58.26 [4.99-441.5] | 7.15 [4.99-145.1] | 27.23 [4.99-186.7] | 0.255 | 0.218285 | 1 | 1 | 0.231 | 0.31333 | 0.31333 | 0.749098 |

| *Cytokines* ***n patients=95 n cytokines= 35*** | *HC (pg/ml) median [IQR]* ***n=30*** | *Immune in pg/ml [Median IQR] n=25* | *Infectious in pg/ml [Median IQR] n=18* | *Idiopathic in pg/ml [Median IQR] n=21* | *P Value for general test of 4 HC vs group* | *Pairwise comparisons p.value (HC vs group)* | | | *P value for General test of disease group vs disease groups* | *Pairwise comparisons p value (disease group vs disease group)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *HC Immune* | *HC infectious* | *HC Idiopathic* | | *Immune infectious* | *Immune Idiopathic* | *Infectious idiopathic* |
| IL-4 | 3.992 [1.973-6.161] | 2.87 [1.92-7.414] | 2.992 [2.192-4.915] | 3.114 [1.701-7.389] | 0.972 | 1 | 1 | 1 | 0.994 | 0.963377 | 0.963377 | 0.963377 |
| IL-5 | 3.36 [1.596-6.295] | 2.397[0.9248-5.564] | 1.299[0.5518-2.977] | 2.755 [1.357-4.706] | **0.0345*** | 0.647846 | **0.015152*** | 1 | 0.0824 | 0.163836 | 0.430633 | 0.085070 |
| IL-6 | 2.242 [1.043-4.389] | 2.508 [0.3053-7.498] | 2.18 [0.8124-3.744] | 4.285 [1.582-7.954] | 0.47 | 1 | 1 | 0.575459 | 0.45 | 0.500167 | 0.500167 | 0.500167 |
| IL-8 | 30.31 [11.27-94.7] | 38.65 [21.55-340.1] | 25.93 [12.96-123] | 44.08 [11.18-181.8] | 0.547 | 0.500995 | 1 | 1 | 0.529 | 0.635555 | 0.635555 | 0.793606 |
| IP-10 | 92.24 [71.35-137.9] | 115.9 [87.57-168.2] | 114.8 [67.32-190.3] | 223.7 [102.4-285.1] | **0.0106*** | 0.677114 | 1 | **0.00298**** | **0.0315*** | 0.723817 | **0.036043*** | **0.036043*** |
| I-TAC | 49.01 [30.79-89.81] | 58.94 [41.83-109.9] | 67.33 [47.14-83.37] | 130.3 [68.55-189.8] | **0.000989***** | 0.446319 | 0.71608 | **0.000182***** | **0.000926****** | 0.873667 | **0.014747*** | **0.014747*** |
| MCP-1 | 572.5 [417.5-754.5] | 550.2 [368.2-865.2] | 449.1 [275.9-496.4] | 621.7 [428.1-779.6] | 0.0695 | 1 | 0.092605 | 1 | **0.0413*** | **0.044169*** | 0.952142 | **0.044169*** |
| MIG | 2609 [1825-3752] | 3686 [2195-6265] | 3039 [2250-6519] | 6158 [2668-9705] | **0.017*** | 0.186629 | 0.625668 | **0.005102**** | 0.19 | 0.765785 | 0.189583 | 0.189583 |

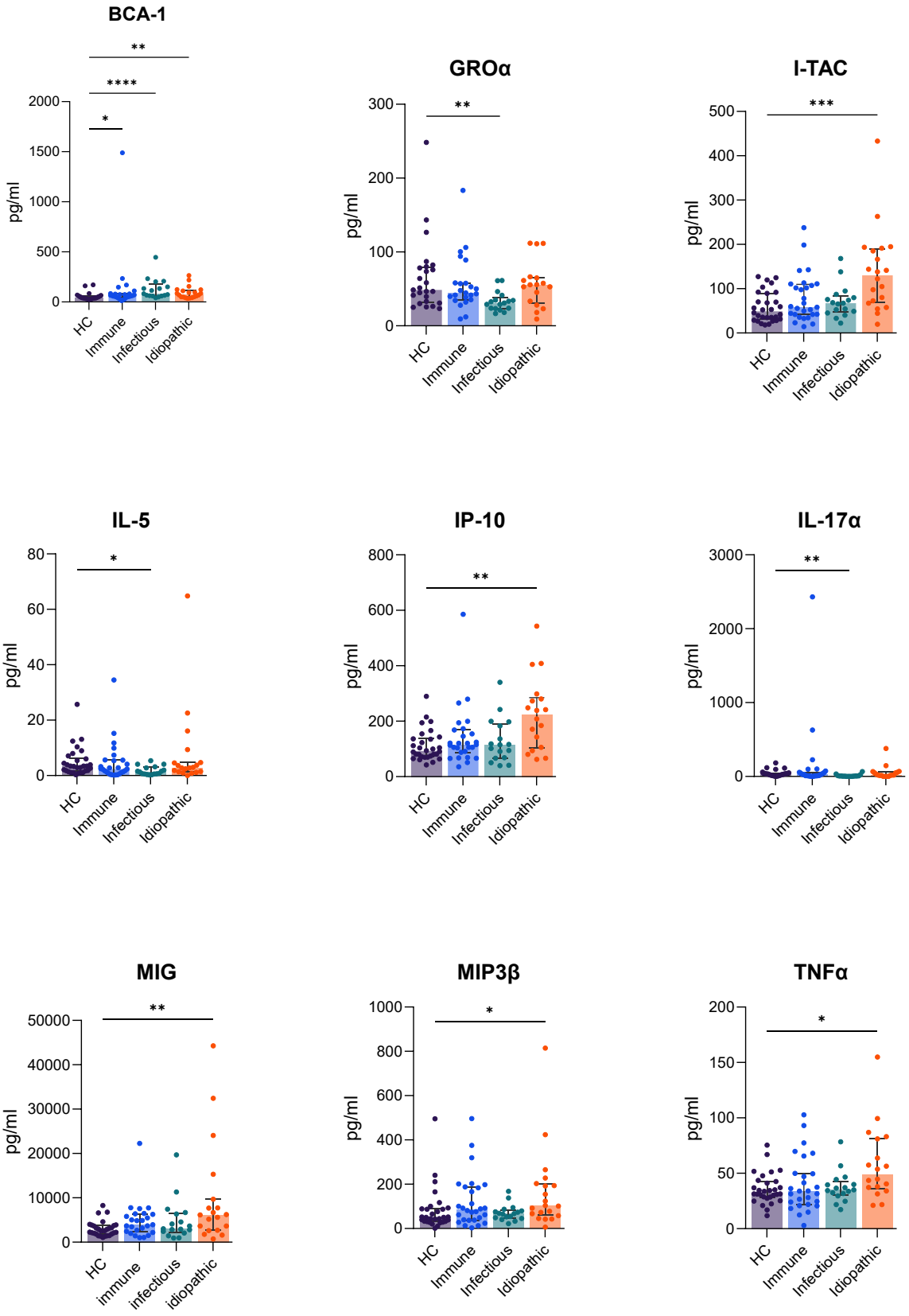
| *Cytokines* ***n patients=95 n cytokines= 35*** | *HC (pg/ml) median [IQR]* ***n=30*** | *Immune in pg/ml [Median IQR] n=25* | *Infectious in pg/ml [Median IQR] n=18* | *Idiopathic in pg/ml [Median IQR] n=21* | *P Value for general test of 4 HC vs group* | *Pairwise comparisons p.value (HC vs group)* | | | *P value for General test of disease group vs disease groups* | *Pairwise comparisons p value (disease group vs disease group)* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *HC Immune* | *HC infectious* | *HC Idiopathic* | | *Immune infectious* | *Immune Idiopathic* | *Infectious idiopathic* |
| MIP-1α | 30.29 [17.7-46.13] | 25.02 [10.82-65.87] | 15.93 [6.507-37.8] | 35.42 [7.479-64.7] | 0.669 | 1 | 1 | 1 | 0.591 | 0.579888 | 0.985489 | 0.579888 |
| MIP-1β | 48.97 [36.47-61.81] | 54.91 [39.38-89.33] | 48.61 [35.23-75.05] | 63.94 [37.28-95.22] | 0.326 | 0.472111 | 1 | 0.365864 | 0.546 | 0.563927 | 0.822727 | 0.563927 |
| MIP-3β | 50.53 [32.01-89.91] | 85.14 [37.98-187] | 131.5 [63.7-167.6] | 102.8 [60.18-200.15] | **0.0258*** | 0.278757 | 0.056469 | **0.021905*** | 0.543 | 0.707093 | 0.707093 | 0.790641 |
| SDF-1 | 4748 [3857-5660] | 5283 [3985-6185] | 4684 [4068-5435] | 5366 [3819-6871] | 0.535 | 0.756737 | 1 | 0.628388 | 0.692 | 0.784795 | 0.784795 | 0.784795 |
| TARC | 101.7 [62.15-167.1] | 131.9 [104.8-223.9] | 116.1 [63.26-207.4] | 127.1 [97.97-205.1] | 0.146 | 0.078480 | 1 | 0.397987 | 0.551 | 0.636736 | 0.636736 | 0.636736 |
| TNF-α | 32.57 [28.36-42.83] | 33.93 [21.91-49.95] | 35 [30.67-42.2] | 49.23 [36.42-81.51] | **0.0266*** | 1 | 1 | **0.013974** | **0.0396*** | 0.868190 | **0.049632*** | 0.067531 |
| VEGF- α | 329.1 [173.2-680.1] | 339 [248.1-509.2] | 305.1 [179-464] | 290.2 [108-610.5] | 0.9 | 1 | 1 | 1 | 0.75 | 0.858017 | 0.858017 | 0.901324 |

***Table 4.1 Concentrations of all 35 cytokines/chemokines are shown as median with IQR***. *Kruskal Wallis for general comparisons then and post-hoc Dunn's for group comparisons with Bonferroni correction for multiple comparisons or post-hoc Benjamini-Hochberg with Bonferroni correction were used for groups comparisons, as indicated. Abbreviations: A Proliferation-Inducing Ligand (APRIL), B Cell-Activating Factor (BAFF), B Cell-Attracting Chemokine 1 (BCA-1, also known as CXCL13), Eosinophil Chemotactic Protein (Eotaxin, or CCL11), Granulocyte Colony-Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), Growth-Regulated Oncogene-alpha (GRO-α, or CXCL1), HC (Healthy Control), Interferon-alpha 2 (IFN-α2), Interferon-gamma (IFN-γ), Interleukin-1 beta (IL-1β), Interleukin-1 receptor antagonist (IL-1Rα), Interleukin-10 (IL-10), Interleukin-12 subunit p70 (IL-12p70), Interleukin-13 (IL-13), Interleukin-15 (IL-15), Interleukin-17 alpha (IL-17α), Interleukin-2 (IL-2), Interleukin-21 (IL-21), Interleukin-22 (IL-22), Interleukin-23 (IL-23), Interleukin-4 (IL-4), Interleukin-5 (IL-5), Interleukin-6 (IL-6), Interleukin-8 (IL-8, or CXCL8), Interferon gamma-induced protein 10 (IP-10, or CXCL10), Interferon-inducible T-cell alpha chemoattractant (I-TAC, or CXCL11), Monocyte Chemoattractant Protein-1 (MCP-1, or CCL2), Monokine Induced by Gamma Interferon (MIG, or CXCL9), Macrophage Inflammatory Protein-1 alpha (MIP-1α, or CCL3), Macrophage Inflammatory Protein-1 beta (MIP-1β, or CCL4), Macrophage Inflammatory Protein-3 beta (MIP-3β, or CCL19), Stromal Cell-Derived Factor 1 (SDF-1, or CXCL12), Thymus and Activation-Regulated Chemokine (TARC, or CCL17), Tumor Necrosis Factor-alpha (TNF-α), and Vascular Endothelial Growth Factor A (VEGF-A).*

As shown in Table 4.1 and Figure 4.1 the following cytokines and chemokines differed significantly between HC and any of the three uveitis aetiologies: BCA-1 was lower in HC compared with immune-mediated uveitis (HC: median [IQR] 40.4 pg/ml [33.41-58.82]; immune-mediated: 62.66 pg/ml[45.61-80.31]. p= 0.049), infectious uveitis (82.61 pg/ml [60.65-176.7], p=0.00006), and idiopathic uveitis (77.64 pg/ml [49.89-120], p=0.0023).

GRO-α showed higher concentrations in HC compared with infectious uveitis (HC: 48.75 pg/ml [31.96-79.32]; infectious uveitis: 30.91 [23.68-38.17], p= 0.0071). IL-5 concentrations were lower in infectious uveitis compared to healthy control levels (infectious uveitis: 1.299 pg/ml [0.5518-2.977]; HC: 3.36 pg/ml [1.596-6.295], p= 0.015). IL-17α concentration was higher in HC compared with infectious uveitis (HC: 32.93 ng/ml [14.19-42.57]; infectious: 5.559 ng/ml [2.444-10.78], p= 0.0017).

Five cytokines were found to have higher serum concentrations in the idiopathic uveitis group compared with HC: I-TAC (idiopathic uveitis: 130.3 pg/ml [68.55-189.8]; HC: 49.01 pg/ml [30.79-89.81], p= 0.000182), MIG (idiopathic uveitis : 6158 pg/ml [2668-9705]; HC: 2609 pg/ml [1825-3752], p= 0.0051), MIP-3β (idiopathic uveitis : 102.8 pg/ml [60.18-200.15]; HC: 50.53 pg/ml [32.01-89.91], p= 0.02195), IP-10 (idiopathic uveitis: 223.7 pg/ml [102.4-285.1]; HC: 92.24 pg/ml [71.35-137.9], p= 0.00298), and TNF-α (idiopathic uveitis: 49.23 pg/ml [36.42-81.51]; HC:32.57 pg/ml [28.36-42.83], p= 0.013974).


BCA-1
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Infectious
Idiopathic
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400
200
0
pg/ml
HC
Immune
Infectious
Idiopathic
IL-17α
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3000
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pg/ml
HC
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MIG
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50000
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pg/ml
HC
Immune
Infectious
Idiopathic

*Figure 4.1: Comparison of serum cytokine/chemokine levels in Healthy controls (HC) and uveitis patients*
*The concentrations of nine relevant cytokines/chemokines are shown as bar graphs (median with IQR) of HC (n=30, dark purple) immune-mediated uveitis (n=39, blue), infectious uveitis (n=24, green) and idiopathic uveitis patients (n=32, orange) Asterisks indicate : * : p<0.05, ** : p<0.01, *** : p<0.001, **** : p<0.0001 Abbreviations: BCA-1 (B-cell activator type 1), GRO-α (Growth-Regulated Oncogene-alpha, also known as CXCL1), IL-2 (Interleukin-2), IL-5 (Interleukin-5), IL-17a (Interleukin-17A), IP-10 (Interferon Gamma-Induced Protein 10, also known as CXCL10), ITAC (Interferon-Inducible T-cell Alpha Chemoattractant, also known as CXCL11), MIG (Monokine Induced by Gamma Interferon, also known as CXCL9), MIP-3b (Macrophage Inflammatory Protein-3 beta, also known as CCL19), TNF-alpha (Tumor Necrosis Factor-alpha).*

## 4.3 Cytokine and chemokine profiles in immune-mediated, infectious and idiopathic uveitis

Next, we compared the serum concentrations of cytokines and chemokines between immune, infectious and idiopathic uveitis patients. Serum concentrations are demonstrated in Table 4.1, and relevant cytokines and chemokines are displayed in Figure 4.2.

Several cytokines/chemokines were significantly different between the three uveitis groups. The immune-mediated and idiopathic uveitis groups had higher concentrations of GRO-α in comparison to the infectious group (immune-mediated: median (IQR) 44.7 pg/ml [35.35-58.13] infectious uveitis: 30.91 pg/ml [23.68-38.17], p= 0.0188; and idiopathic uveitis: 55.51 pg/ml [30.79-65.73], p=0.0188).

IL-17α concentrations were significantly higher in the immune group, with the median immune concentration being 25.95 pg/ml, (IQR 9.689-55.17) compared to the infectious concentration (5.559 ng/ml, 2.444-10.78, p-value immune-infectious= 0.009755). Idiopathic uveitis also had an elevated IL-17α concentration compared to infectious uveitis (idiopathic 18.31 ng/ml, IQR 10.16-59.27, p=0.016723).

The highest IP-10 levels were observed idiopathic uveitis group (223.7 pg/ml, 102.4-285.1), compared to the immune-mediated (115.9 pg/ml, 87.57-168.2, p=0.0360) and infectious group (114.8 pg/ml, 67.32-190.3, p=0.0360).

Additionally, I-TAC levels were significantly higher in idiopathic uveitis when compared to infectious and immune uveitis (immune uveits: 58.94 [41.83-109.9] idiopathic uveitis: 130.3 pg/ml [68.55-189.8]; infectious uveitis: 67.33 pg/ml [47.14-83.37], p=0.0147). MCP-1 levels were higher in the immune-mediated and idiopathic group compared to the infectious group (immune-mediated: 550.2 pg/ml [368.2-865.2]; infectious: 449.1 pg/ml [275.9-496.4], idiopathic: 621.7 pg/ml [428.1-779], p-value immune-infectious and infectious-idiopathic both = 0.044). Lastly, TNF-α was significantly lower in the immune-mediated group compared to the idiopathic group (immune-mediated: 33.93 pg/ml [21.91-49.95]; idiopathic: 49.23[36.42-81.51], p=0.0496)

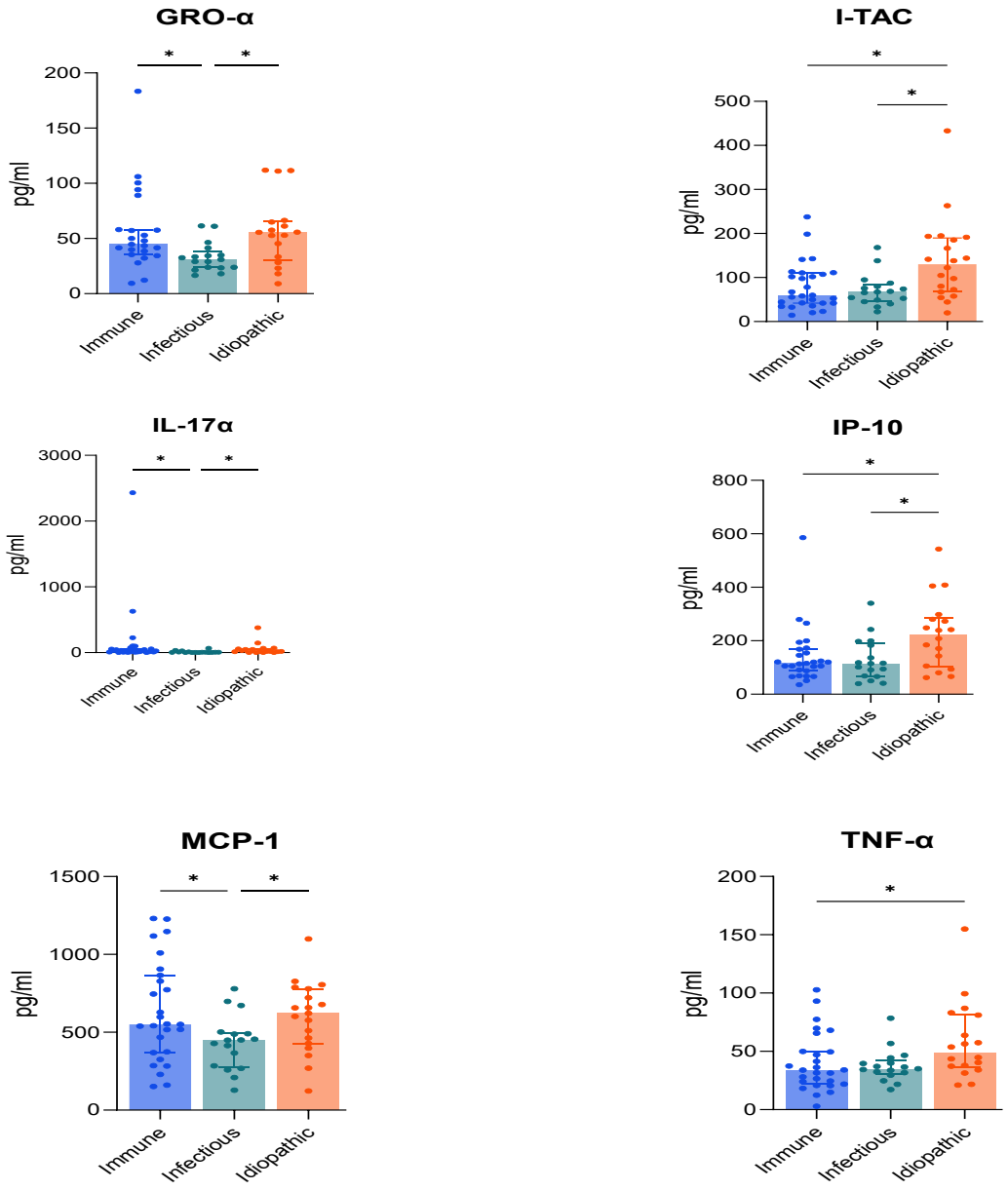


*Figure 4.2 : Cytokine/Chemokine levels of immune, infectious and idiopathic uveitis patients. Bar graphs (median; IQR) of significantly different cytokines. Kruskal-Wallis test with post-hoc Dunn's test and Benjamini-Hochberg correction (significance.*: p<0.05). Abbreviations: GRO-α (Growth-Regulated Oncogene-alpha), I-TAC (IFN-inducible T-cell Alpha Chemoattractant), IL-17α (Interleukin-17 alpha), IP-10 (Interferon gamma-induced Protein 10), MCP-1 (Monocyte Chemoattractant Protein-1), and TNF-α (Tumor Necrosis Factor-alpha).*

## 4.4 Live-cell based assay for MOG Antibody detection.

We used a live cell-based analysis to investigate if any of the serum samples were MOG antibody positive. None of the 67 samples tested returned a clear positive result.

## 4.5 Cluster analysis of cytokine and chemokine levels

After identifying relevant cytokines and chemokines that were significantly different in the serum of HC and the three uveitis aetiologies, we proceeded to characterise the cytokine/chemokine profile more thoroughly. Firstly, we developed heatmaps of Spearman correlation coefficients of cytokines/ chemokines and applied hierarchical clustering in HC, immune, infectious and idiopathic uveitis, respectively (Figures 4.3 – 4.6). Strong correlations between cytokines/ chemokines are depicted in red colour, whereas negative correlations are shown in blue. A white colour indicates no correlation between the respective cytokines/ chemokines. Dendrograms show the pattern formation as a result of hierarchical clustering. HCs (Figure 4.3) only showed little correlation between cytokines/ chemokines, whereas in the three uveitis groups (figure 4.4-4.6), distinct pattern were observed. Especially in the idiopathic uveitis group (Figure 4.6), strong correlations of predominantly pro-inflammatory cytokines/ chemokines were observed, highlighting a potential underlying inflammatory cause.

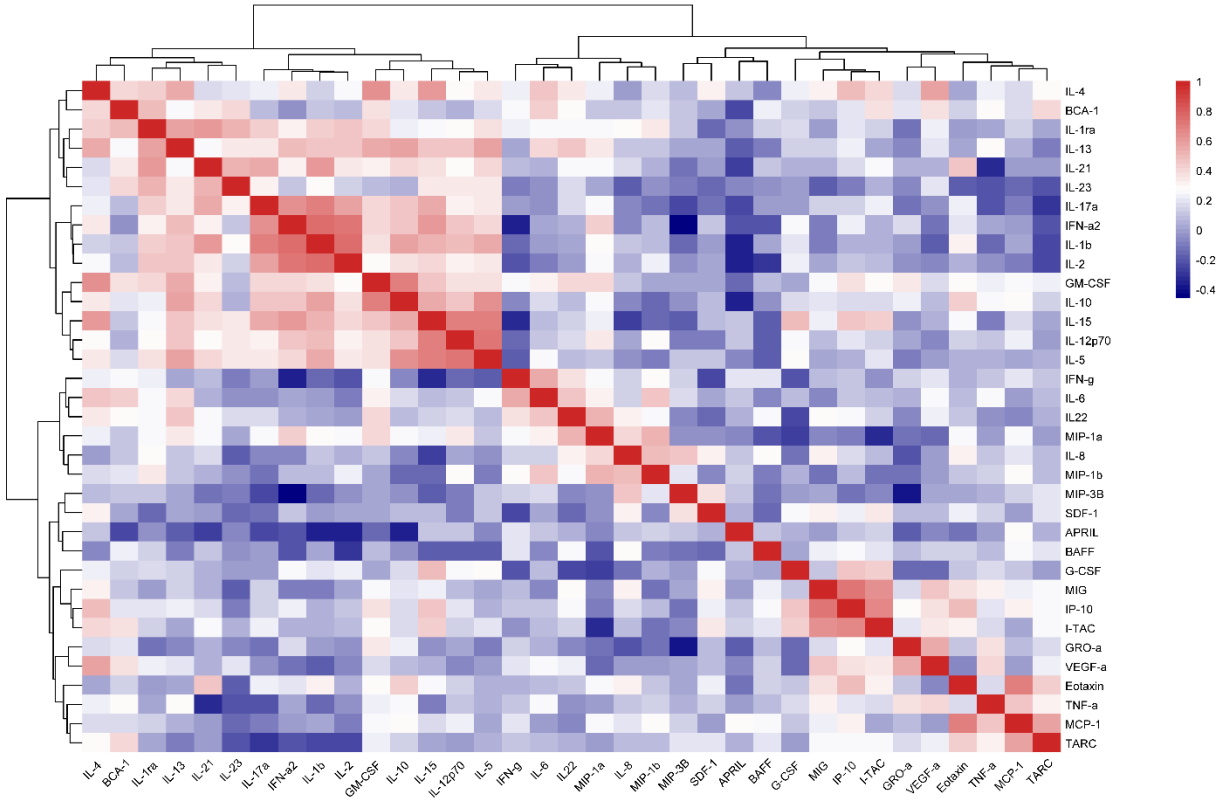


***Figure 4.3:*** *Heatmap showing Spearman correlation coefficients (positive correlation = red, negative correlation = blue, no correlation = white) and hierarchical clustering dendrograms (left y-axis, top x-axis) of 35 cytokines and chemokines (right y-axis, bottom x-axis) in the serum of healthy controls (n=23).*

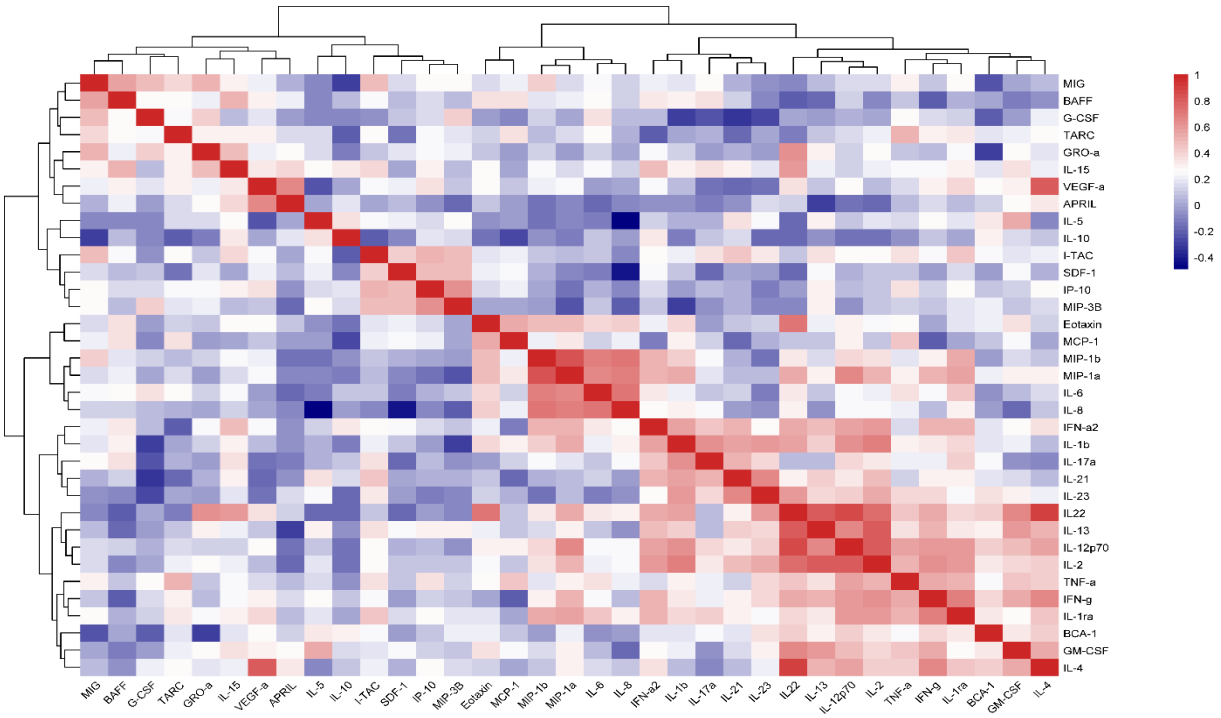

***Figure 4.4:*** *Heatmap showing Spearman correlation coefficients (positive correlation = red, negative correlation = blue, no correlation = white) and hierarchical clustering dendrograms (left y-axis, top x-axis) of 35 cytokines and chemokines (right y-axis, bottom x-axis) in the serum of immune-mediated uveitis patients (n=23).*

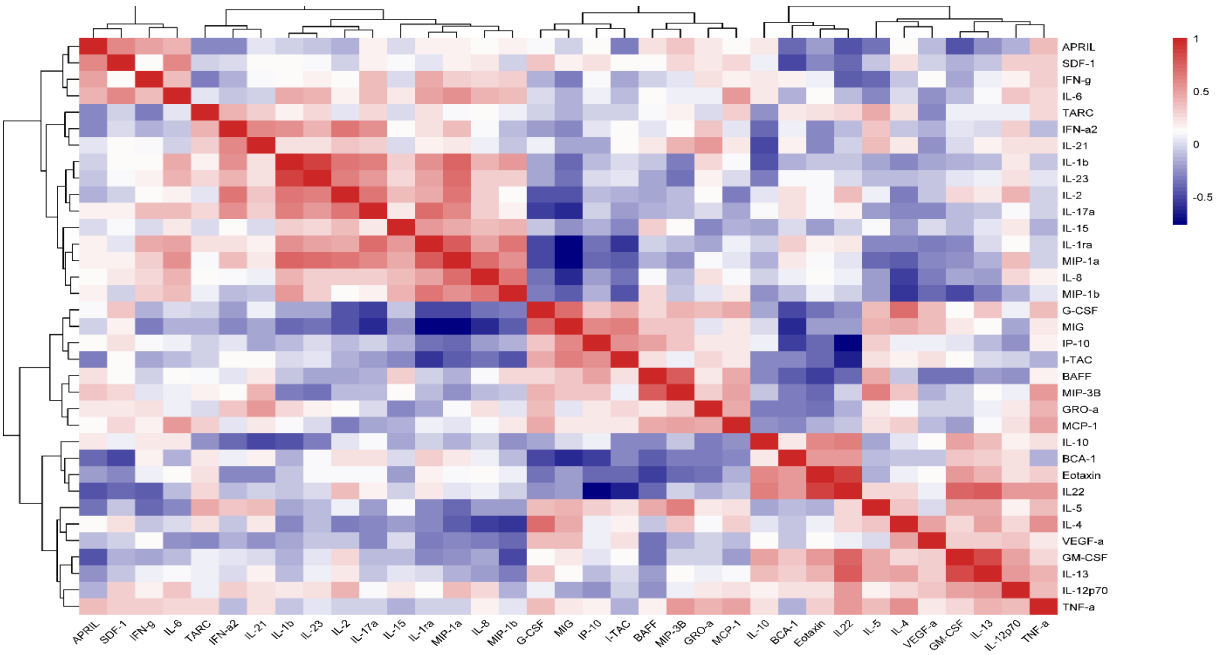


***Figure 4.5:*** *Heatmap showing Spearman correlation coefficients (positive correlation = red, negative correlation = blue, no correlation = white) and hierarchical clustering dendrograms (left y-axis, top x-axis) of 35 cytokines and chemokines (right y-axis, bottom x-axis) in the serum of infectious uveitis patients (n=15).*

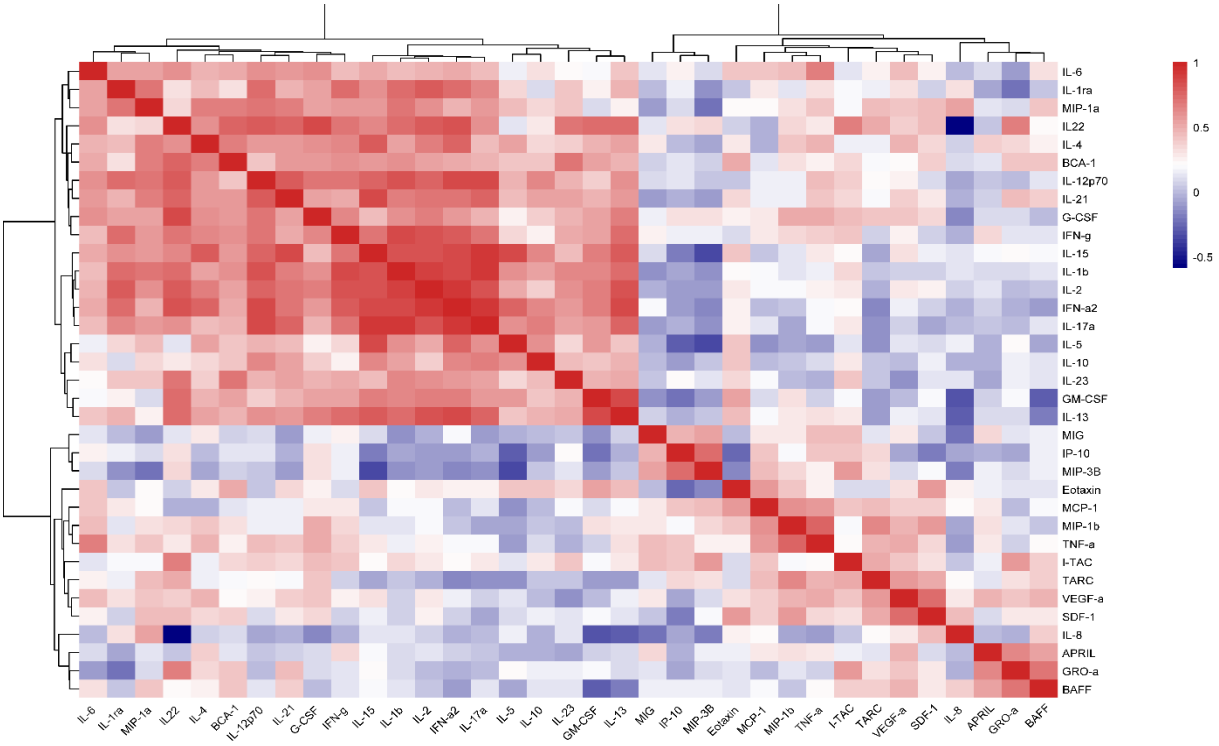

***Figure 4.6:*** *Heatmap showing Spearman correlation coefficients (positive correlation = red, negative correlation = blue, no correlation = white) and hierarchical clustering dendrograms (left y-axis, top x-axis) of 35 cytokines and chemokines (right y-axis, bottom x-axis) in the serum of idiopathic uveitis patients (n=15).*

Next, principal component analysis (PCA) was performed, to study whether the 35 cytokines and chemokines show visual clustering in two dimensions. PCA was done for HC as well as the immune, infectious and idiopathic uveitis groups; as well as the three uveitis groups alone.

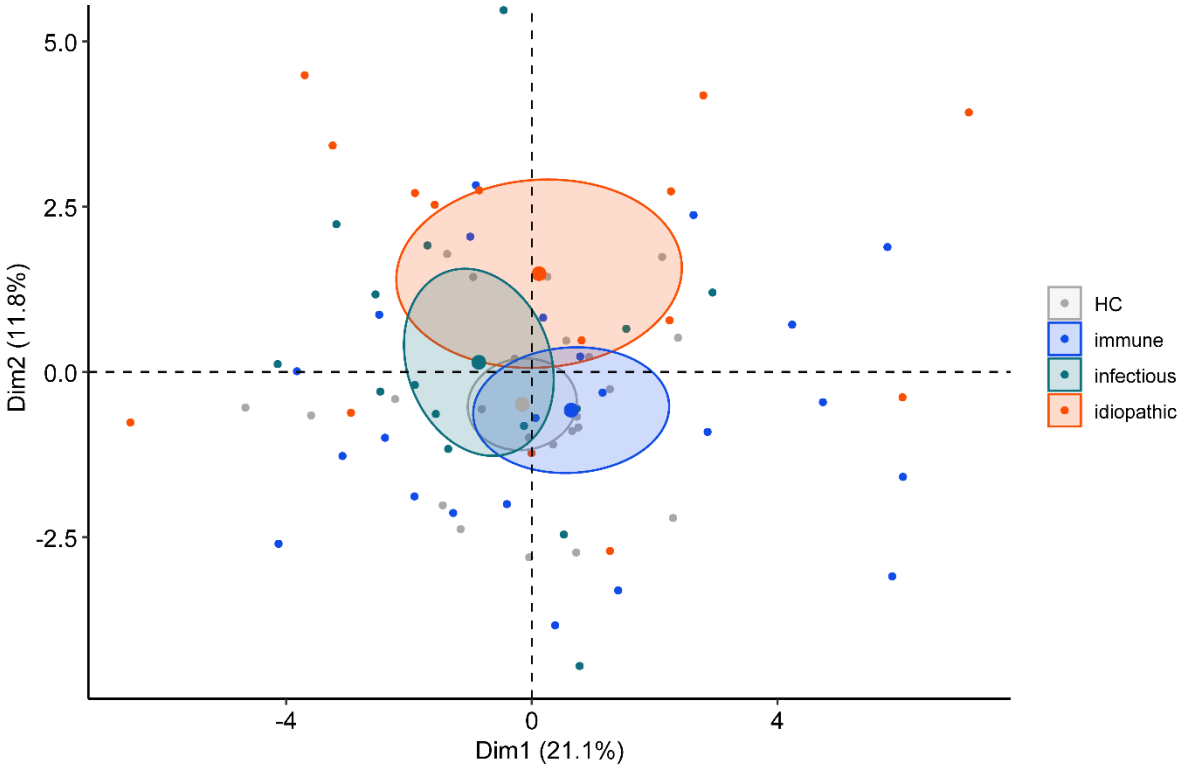


*Figure 4.7: Principle component analysis of 35 cytokines/chemokines in healthy controls (HC, n=23, grey), immune-mediated (n=23, blue), infectious uveitis (n=15, green) and idiopathic uveitis (n=15, orange). Individual points and confidence ellipses are shown.*

Figure 4.7 shows the PCA of HC and the three uveitis groups including all 35 cytokines/chemokines. Confidence ellipses indicated differential clustering between the uveitis

groups and HC (Permutational Multivariate Analysis of Variance (PERMANOVA) Pr(>F)=0.045; pairwise comparisons all non-significant).

A more refined analysis including only the cytokines/chemokines with significant differences between HC and any of the uveitis groups (as discussed in Chapter 4.2 and visualised in Figure 4.1) in an overall Kruskal-Wallis test was undertaken next. As presented in Figure 4.8, after limiting the included cytokines/chemokines to BCA-1, GRO-α, I-TAC, IL-17a, IL-5, IP-10, MIG, MIP-3β, and TNF-α, a better separation between groups could be achieved (Figure 4.8A, PERMANOVA Pr(>F)=0.001; pairwise PERMANOVA: HC-immune p=0.679, R2=0.015; HC-infectious p=0.006, R2=0.198; HC-idiopathic p=0.006, R2=0.125; immune-infectious p=0.03, R2=0.092; immune-idiopathic p=0.068, R2=0.063; infectious-idiopathic p=0.02, R2=0.113). The relative contribution of the most differentiating cytokines/chemokines for driving this clustering are highlighted in Figure 4.8B.

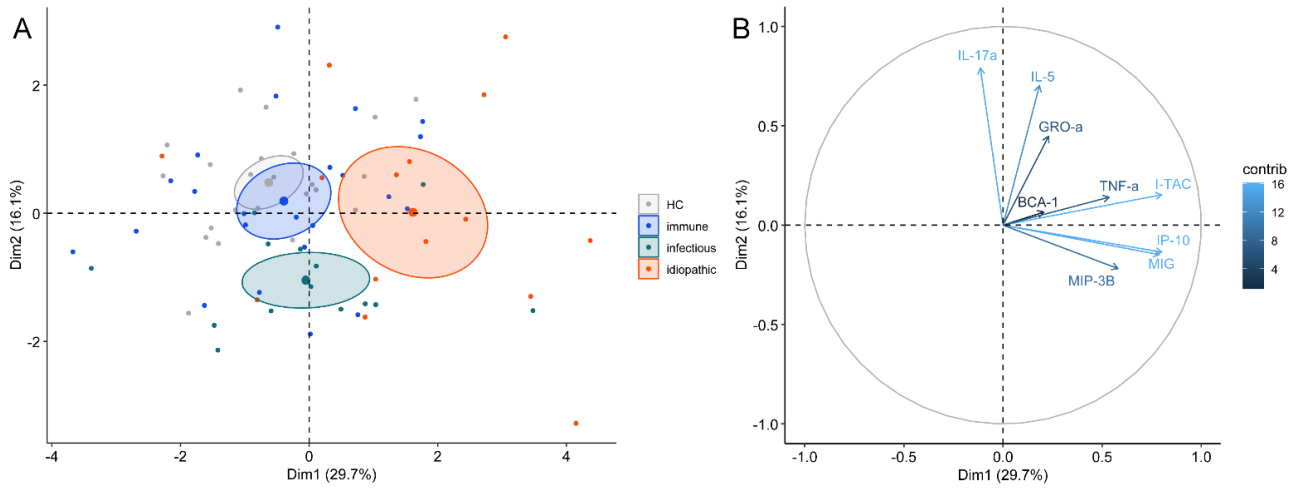


***Figure 4.8: A:*** *Principal component analysis of 9 relevant cytokines/chemokines in healthy controls (HC, n=23, grey), immune uveitis (n=23, blue), infectious uveitis (n=15, green) and idiopathic uveitis (n=15, orange). Individual points and confidence ellipses are shown.* ***B:*** *Plot showing the relative contribution of each cytokines/chemokine for driving clustering into either dimension.*

Figure 4.9 shows a PCA including all 35 cytokines/chemokines, and evaluating differences only between the three uveitis groups, with healthy controls excluded. No significant differences between immune, infectious, and idiopathic uveitis (PERMANOVA Pr(>F)=0.099; pairwise comparisons all non-significant) were observed.

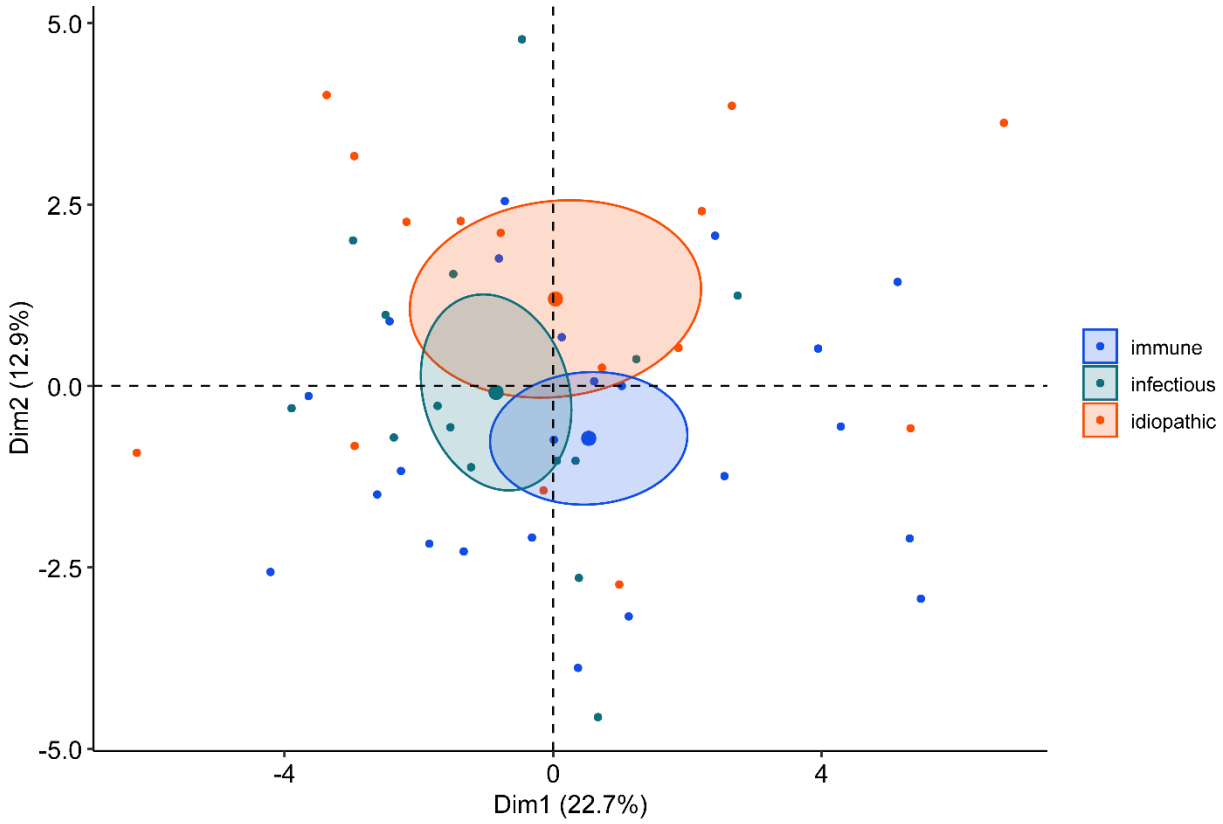


***Figure 4.9:*** *Principal component analysis of 35 cytokines/chemokines in immune uveitis (n=23, blue), infectious uveitis (n=15, green) and idiopathic uveitis (n=15, orange). Individual points and confidence ellipses are shown.*

However, a focused PCA and PERMANOVA analysis of cytokines/chemokines between the three uveitis groups only, allowed a more selective approach and demonstrated a more pronounced separation between groups. After limiting the included cytokines/chemokines to the six relevant ones (GRO-α, I-TAC, IL-17a, IP-10, MCP-1, and TNF-α) (see Chapter 4.3, Figure 4.2) that showed significant differences in the overall Kruskal-Wallis test between groups, the PCA demonstrated distinct clusters (Figure 4.10A; PERMANOVA Pr(>F)=0.002; pairwise PERMANOVA: immune-infectious p=0.01, R2=0.134; immune-idiopathic p=0.042, R2=0.073; infectious-idiopathic p=0.003, R2=0.164). The relative contribution of the most differentiating cytokines/chemokines for driving this clustering are highlighted in Figure 4.10B.

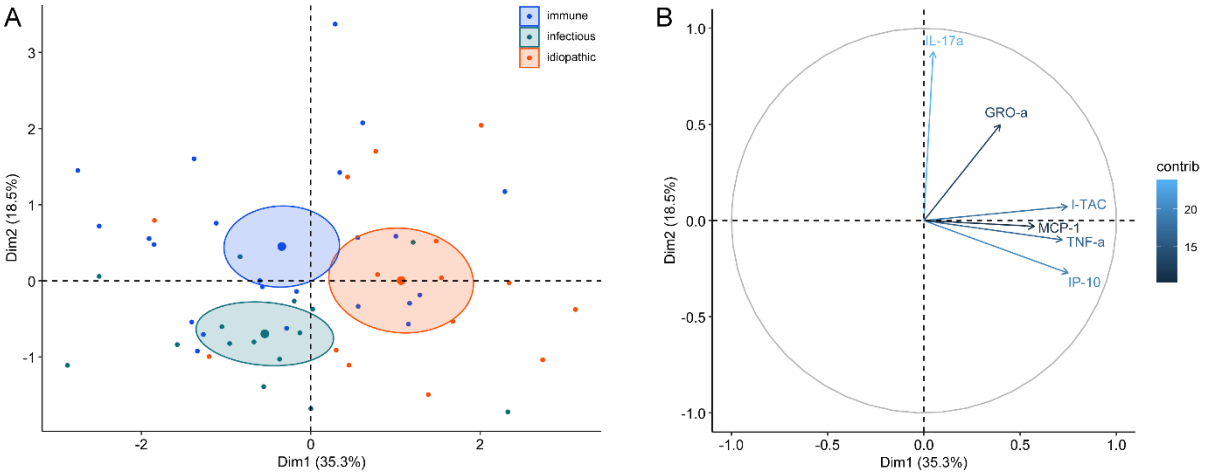


***Figure 4.10: A:*** *Principal component analysis of 6 relevant cytokines/chemokines in immune uveitis (n=23, blue), infectious uveitis (n=15, green) and idiopathic uveitis (n=15, orange). Individual points and confidence ellipses are shown.* ***B:*** *Plot showing the relative contribution of each cytokines/chemokine for driving clustering into either dimension.*

These results suggest that the infectious and immune-mediated uveitis aetiologies studied here show a distinct peripheral cytokine/chemokine profile which may be of diagnostic utility. However, the idiopathic uveitis cohort seems to be a more separate group, with significant heterogeneity.

# Chapter 5 Discussion and conclusions

To the best of our knowledge, this is the first study to integrate a prospective, non-invasive cytokine/chemokine evaluation of acute uveitis across all major aetiological groups—immune-mediated, infectious, and idiopathic—with a healthy control comparison. This study features longitudinal follow-up with comprehensive assessment of both clinical and immunopathological outcomes, allowing for cross-group comparisons. Previous studies investigating cytokines in uveitis focused on retrospective cohorts, required more invasive intrao-ocular samples, investigated only one of these aetiological groups, or evaluated cytokine profiles in one or more specific diseases (171, 184, 185).

## 5.1 Limitations

We defined patients as having acute uveitis as being within 90 days of uveitis onset, in order to ensure standardised recruitment. This could have led to patients being recruited with biosamples collected very early (within a few days of onset and before the commencement of antimicrobial or immune treatment), as well as patients within two months of onset with antimicrobial treatment or immunotherapy already on board. The impact of such treatment on the cytokine and chemokine milieu may need to be recognised. Recruitment was made more challenging in the context of the COVID-19 pandemic at the time of recruitment, which made rapid attendance to clinic and acute sample collection more difficult during periods of lockdown. Regardless, we were able to consistently recruit, and sample consented patients within the defined timeframe, and we collected samples as close to disease onset as possible to mitigate this limitation.

The Kruskal-Wallis test was selected as the most appropriate statistical method for comparing non-parametric data across the multiple independent groups in this study. Recruitment numbers for this project were limited due to constraints imposed by the COVID-19 pandemic and the finite data collection period of the project. The resultant reduction in sample size may have diminished the statistical power to detect smaller effect sizes, and this is acknowledged as a recognised limitation of the study.

Another limitation was the heterogeneity of causes of both immune and infectious uveitis. The pathology of uveitis may be different between different immune-mediated aetiologies, which may present varied cytokine/chemokine profiles. A subgroup analysis by disease would have further elucidated some of these differences, however the numbers of patients with specific disease aetiologies within these broader categories were small, and as a result robust disease specific associations were out of the scope of this study.

## 5.2 Implications of results

In our cohort, patients with immune-mediated uveitis had more relapses and were followed up for longer than idiopathic and infectious uveitis patients, respectively. The immune-mediated patients, as might be intuitively thought, had more frequent systemic autoimmune diagnoses or a family history of autoimmunity in first degree relatives. Furthermore, patients with idiopathic uveitis had the highest rate of ophthalmologic complications, highlighting the real-world challenges of treating an undifferentiated condition symptomatically, without clear aetiological guidance.

Out of the tested visual outcomes, many were similar between diagnostic categories, or did not reach significance. The only finding that was different was the macular thickness at nadir

presentation was higher in the immune-mediated uveitis group compared to the infectious uveitis group. This relative lack of difference in visual parameters between diagnostic categories could be due to the fact that changes in the retinal layer thickness, for example, may be dynamic at different times of the disease process, and depend on how quickly the acute OCT was performed. Regardless, the macular thickness difference at nadir points towards a potential signal looking at the severity of disease by aetiology.

Our data suggests that while certain demographic and clinical features are consistent across uveitis aetiologies, significant differences exist in the clinical course, relapse patterns, systemic associations, and initial macular involvement, highlighting the importance of considering the aetiology for the management of uveitis.

We had previously described the presence of uveitis in patients presenting with acute MOGAD with associated optic neuritis (110). None of the patients in this cohort of 67 patients were MOG antibody positive, which may indicate that this is a relatively uncommon cause of uveitis.

Of our 35 evaluated cytokines, we identified nine cytokines which best differentiated uveitis patients from healthy controls, and six which discriminated well between diagnostic categories of acute uveitis. BCA-1 emerges as a cytokine that is significantly higher in all disease groups than in healthy controls. This cytokine is usually a chemotactic cytokine that attracts B cells (186), suggesting that B cell activation and migration to the otherwise immune-privileged uvea could be a marker of early inflammatory disease.

In keeping with prior studies, our studies demonstrated IL-17, TNF-α, and Th-1 mediated as being useful differentiators. Patients with immune-mediated uveitis had higher IL-17α levels, compared to the infectious and idiopathic groups, suggesting a contribution of the Th-17 pathway in the pathophysiology of immune-mediated uveitis. On the other hand, patients with infectious uveitis had lower IL-17α and lower GROα levels, which both play a role in neutrophil recruitment, suggesting an impairment of neutrophil recruitment in infectious uveitis. Interestingly, the cytokines that were elevated in idiopathic uveitis compared to immune or infectious uveitis (IP-10, TNF-α, and MCP-1) are involved in Th-1 mediated inflammation, suggesting a potentially common underlying disease pathogenesis within patients in this group.

In immune-mediated uveitis, our study demonstrated a strong Th17 skew, marked by elevated IL-17α levels, which aligns with existing literature and partially overlaps with findings from Errera et al., who reported IL-17 elevation in idiopathic cases. Notably, Errera's study did not include a distinct immune-mediated group, highlighting the value of our data in differentiating autoimmune from idiopathic uveitis. In infectious uveitis, we observed lower levels of both IL-17α and GROα, suggesting impaired neutrophil recruitment—an insight not explored in Errera's work. Their focus on toxoplasmosis may explain this difference, as neutrophilic responses could be more suppressed in parasitic than in viral uveitis. The identification of GROα downregulation in our study offers a novel perspective on deficits in innate immunity. In idiopathic uveitis, both Errera's work and our study consistently identified elevated levels of IP-10, TNF-α, and MCP-1, reflecting a Th1-biased inflammatory profile. This convergence reinforces our hypothesis that a shared Th1-mediated mechanism may underlie pathology across idiopathic uveitis cases.

Heat maps of hierarchical clustering demonstrated a fairly separate profile between the three aetiologies and healthy controls. We identified the nine most important contributors of cytokines/chemokines that best differentiated healthy controls from uveitis patients, and the six which best differentiated between uveitis aetiologies. This allowed for clear separation of infectious and immune-mediated uveitis in principle component analysis using a limited panel of cytokines and chemokines, which may enable translation for clinical and diagnostic utility, while being targeted and cost-effective.

Interestingly, patients with idiopathic uveitis were fairly separate from both immune-mediated and infectious uveitis groups, suggesting that some patients with uveitis classified as idiopathic may have a distinct underlying aetiology (for example, genetic etc). Alternatively, the group currently classified as idiopathic is likely to be made up of a fairly heterogenous group of disorders that do not cluster well together.

Regardless, using the current profile of 9 key cytokines/chemokines identified in our study may be used to raise the clinical index of suspicion for an immune-mediated vs infectious cause.

## 5.3 Conclusions and future directions

Our study provides a comprehensive clinical and cytokine/chemokine analyses of prospectively recruited patients with acute immune-mediated, infectious, and idiopathic uveitis. We identified a refined panel which provided good discrimination between immune-mediated and infectious aetiologies, and which could assist clinicians in the diagnostic work up of patients where the aetiology of uveitis is unclear.

Future research priorities include replication of the findings of this study in larger and more geographically diverse cohorts, as well as an understanding of how cytokine/chemokine profiles may vary longitudinally over a patient's disease course. Patients with idiopathic uveitis may have more heterogenous aetiologies, and based on our results, the role of the innate immune system in these patients should be further evaluated.

# Bibliography

1. Rathinam SR, Namperumalsamy P. Global variation and pattern changes in epidemiology of uveitis. Indian J Ophthalmol. 2007;55(3):173-83.

2. Standring S. Two drawings by Franz Batke in Gray's Anatomy. Clin Anat. 2012;25(7):911.

3. Kim T, Blackmon DM. CHAPTER 9 - Uveitis. In: Palay DA, Krachmer JH, editors. Primary Care Ophthalmology (Second Edition). Philadelphia: Mosby; 2005. p. 139-48.

4. Rosenbaum JT, Becker, M., Smith, J,. Clinical Immunology Principles and Practice-3rd Edition.1085-97.

5. Teo AYT, Betzler BK, Hua KLQ, Chen EJ, Gupta V, Agrawal R. Intermediate Uveitis: A Review. Ocul Immunol Inflamm. 2022:1-20.

6. Harthan JS, Opitz DL, Fromstein SR, Morettin CE. Diagnosis and treatment of anterior uveitis: optometric management. Clin Optom (Auckl). 2016;8:23-35.

7. Dalvin LA, Smith WM. Intraocular manifestations of mycobacterium tuberculosis: A review of the literature. J Clin Tuberc Other Mycobact Dis. 2017;7:13-21.

8. Gupta P, Goyal V, Srivastava AK, Pandit AK, Prasad K. Uveitis, optic neuritis and MOG. Mult Scler J Exp Transl Clin. 2020;6(2):2055217320925107.

9. Acharya NR, Tham VM, Esterberg E, Borkar DS, Parker JV, Vinoya AC, et al. Incidence and prevalence of uveitis: results from the Pacific Ocular Inflammation Study. JAMA Ophthalmol. 2013;131(11):1405-12.

10. Garcia-Aparicio A, Garcia de Yebenes MJ, Oton T, Munoz-Fernandez S. Prevalence and Incidence of Uveitis: A Systematic Review and Meta-analysis. Ophthalmic Epidemiol. 2021;28(6):461-8.

11. Gritz DC, Schwaber EJ, Wong IG. Complications of Uveitis: The Northern California Epidemiology of Uveitis Study. Ocul Immunol Inflamm. 2018;26(4):584-94.

12. Guo D, Liu J, Gao R, Tari S, Islam S. Prevalence and Incidence of Optic Neuritis in Patients with Different Types of Uveitis. Ophthalmic Epidemiol. 2018;25(1):39-44.

13. Umazume A, Ohguro N, Okada AA, Namba K, Sonoda KH, Tsuruga H, et al. Prevalence and incidence rates and treatment patterns of non-infectious uveitis in Japan: real-world data using a claims database. Jpn J Ophthalmol. 2021;65(5):657-65.

14. Tan J, Zhu E, Hart C, Rogers S, Lim LL. Epidemiology of Anterior Uveitis in Metropolitan Melbourne. Invest Ophth Vis Sci. 2018;59(9).

15. Julian LK, Balfour G, Forgues RF, de Smet MD, Suburo A. Uveitis Patterns and Severity: An Epidemiologic Study from a Tertiary Care Private Referral Center in Buenos Aires, Argentina. Ocul Immunol Inflamm. 2022:1-7.

16. Rodriguez A, Calonge M, Pedroza-Seres M, Akova YA, Messmer EM, D'Amico DJ, et al. Referral patterns of uveitis in a tertiary eye care center. Arch Ophthalmol. 1996;114(5):593-9.

17. Hart CT, Zhu EY, Crock C, Rogers SL, Lim LL. Epidemiology of uveitis in urban Australia. Clinical & Experimental Ophthalmology. 2019;47(6):733-40.

18. Rao NA. Uveitis in developing countries. Indian Journal of Ophthalmology. 2013;61(6):253-4.

19. Durrani OM, Tehrani NN, Marr JE, Moradi P, Stavrou P, Murray PI. Degree, duration, and causes of visual loss in uveitis. Br J Ophthalmol. 2004;88(9):1159-62.

20. Suttorp-Schulten MS, Rothova A. The possible impact of uveitis in blindness: a literature survey. Br J Ophthalmol. 1996;80(9):844-8.

21. Tan H, Feng X, Yang P. Association between uveitis onset and economic development in mainland China. BMC Public Health. 2023;23(1):1711.

22. ET EC, Zierhut M. Vision Loss in Uveitis. Ocul Immunol Inflamm. 2021;29(6):1037-9.

23. Smith JR, Rosenbaum JT. Neurological concomitants of uveitis. Br J Ophthalmol. 2004;88(12):1498-9.

24. Dick AD, Tundia N, Sorg R, Zhao C, Chao J, Joshi A, et al. Risk of Ocular Complications in Patients with Noninfectious Intermediate Uveitis, Posterior Uveitis, or Panuveitis. Ophthalmology. 2016;123(3):655-62.

25. Krishna U, Ajanaku D, Denniston AK, Gkika T. Uveitis: a sight-threatening disease which can impact all systems. Postgrad Med J. 2017;93(1106):766-73.

26. Miserocchi E, Fogliato G, Modorati G, Bandello F. Review on the Worldwide Epidemiology of Uveitis. European Journal of Ophthalmology. 2013;23(5):705-17.

27. Lee SY, Mesfin FB. Blindness. StatPearls. Treasure Island (FL)2023.

28. Austroads. Vision and Eye Disorders – Medical Standards for Licensing. 2022.

29. Kaiser PK. Prospective evaluation of visual acuity assessment: a comparison of snellen versus ETDRS charts in clinical practice (An AOS Thesis). Trans Am Ophthalmol Soc. 2009;107:311-24.

30. Villalobos-Perez A, Reyes-Guanes J, Munoz-Ortiz J, Estevez-Florez MA, Ramos-Santodomingo M, Balaguera-Orjuela V, et al. Referral Process in Patients with Uveitis: A Challenge in the Health System. Clin Ophthalmol. 2021;15:1-10.

31. Seve P, Cacoub P, Bodaghi B, Trad S, Sellam J, Bellocq D, et al. Uveitis: Diagnostic work-up. A literature review and recommendations from an expert committee. Autoimmun Rev. 2017;16(12):1254-64.

32. Tucker W, Graham E, Murray P, Stanford M. Idiopathic Intermediate Uveitis: A 25-year study of visual prognosis. Invest Ophth Vis Sci. 2013;54(15):355-.

33. Jabs DA, Nussenblatt RB, Rosenbaum JT, Standardization of Uveitis Nomenclature Working G. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. Am J Ophthalmol. 2005;140(3):509-16.

34. Standardization of Uveitis Nomenclature Working G. Development of Classification Criteria for the Uveitides. Am J Ophthalmol. 2021;228:96-105.

35. Standardization of Uveitis Nomenclature Working G. Classification Criteria for Multiple Sclerosis-Associated Intermediate Uveitis. Am J Ophthalmol. 2021;228:72-9.

36. Rothova A, van Veenedaal WG, Linssen A, Glasius E, Kijlstra A, de Jong PT. Clinical features of acute anterior uveitis. Am J Ophthalmol. 1987;103(2):137-45.

37. Agrawal RV, Murthy S, Sangwan V, Biswas J. Current approach in diagnosis and management of anterior uveitis. Indian J Ophthalmol. 2010;58(1):11-9.

38. Rothova A. Medical treatment of cystoid macular edema. Ocul Immunol Inflamm. 2002;10(4):239-46.

39. Standardization of Uveitis Nomenclature Working G. Classification Criteria for Intermediate Uveitis, Non-Pars Planitis Type. Am J Ophthalmol. 2021;228:159-64.

40. Abraham A, Nicholson L, Dick A, Rice C, Atan D. Intermediate uveitis associated with MS: Diagnosis, clinical features, pathogenic mechanisms, and recommendations for management. Neurol Neuroimmunol Neuroinflamm. 2021;8(1).

41. Ebrahimiadib N, Adelpour F, Ghahari M, Mehrpour M. Intermediate Uveitis Associated with Tattooing of Eyebrows as a Manifestation of Systemic Sarcoidosis: Report of Two Cases. Ocul Immunol Inflamm. 2021;29(5):902-5.

42. Rojas-Carabali W, Reyes-Guanes J, Villabona-Martinez V, Fonseca-Mora MA, de-la-Torre A. Intermediate Uveitis Etiology, Complications, Treatment, and Outcomes in a Colombian Uveitis Referral Center. Clin Ophthalmol. 2021;15:2597-605.

43. Schneider J, Schalenbourg A, Dudler J, Guex-Crosier Y. Intermediate Uveitis in an HLA-B27-Positive Patient Treated with Upadacitinib. Klin Monbl Augenheilkd. 2022;239(4):572-4.

44. McCluskey PJ, Watson PG, Lightman S, Haybittle J, Restori M, Branley M. Posterior scleritis: clinical features, systemic associations, and outcome in a large series of patients. Ophthalmology. 1999;106(12):2380-6.

45. Gupta V, Al-Dhibi HA, Arevalo JF. Retinal imaging in uveitis. Saudi J Ophthalmol. 2014;28(2):95-103.

46. Nussenblatt RB, Palestine AG, Chan CC, Roberge F. Standardization of vitreal inflammatory activity in intermediate and posterior uveitis. Ophthalmology. 1985;92(4):467-71.

47. Rispoli M, Bruno L, ProQuest. Practical Handbook of Fluorescein Angiography. 2nd edition. ed. Delhi: Jaypee Brothers Medical Publishers; 2022.

48. Ness T, Boehringer D, Heinzelmann S. Intermediate uveitis: pattern of etiology, complications, treatment and outcome in a tertiary academic center. Orphanet J Rare Dis. 2017;12(1):81.

49. Uy HS, Yu-Keh E, Chan PS. Posterior Uveitis. Dev Ophthalmol. 2016;55:163-6.

50. Yang P. Posterior Uveitis. Atlas of Uveitis: Diagnosis and Treatment. Singapore: Springer Singapore; 2021. p. 195-201.

51. Ayliffe W. Retinal Vasculitis. Foster CS, Vitale Albert T, editors: Jaypee Brothers Medical Publishers (P) Ltd.; 2013 2013/01/01.

52. Herbort CP. Fluorescein and indocyanine green angiography for uveitis. Middle East Afr J Ophthalmol. 2009;16(4):168-87.

53. Herbort CP, Jr., Mantovani A, Tugal-Tutkun I, Papasavvas I. Classification of Non-Infectious and/or Immune Mediated Choroiditis: A Brief Overview of the Essentials. Diagnostics (Basel). 2021;11(6).

54. Tian M, Zeng G, Tappeiner C, Zinkernagel MS, Wolf S, Munk MR. Comparison of Indocyanine Green Angiography and Swept-Source Wide-Field Optical Coherence Tomography Angiography in Posterior Uveitis. Front Med (Lausanne). 2022;9:853315.

55. Russell K, Griffin, Jardine. FUNDUS PHOTOGRAPHY, FLUORESCEIN ANGIOGRAPHY, AND INDOCYANINE GREEN ANGIOGRAPHY OF TUBERCULOUS CHOROIDITIS [Educational website]. 2017 [Available from: https://morancore.utah.edu/section-09-intraocular-inflammation-and-uveitis/fundus-photography-fluorescein-angiography-and-indocyanine-green-angiography-of-tuberculous-choroiditis/.

56. Orazbekov L, Yasukawa T, Hirano Y, Ogura S, Usui H, Nozaki M, et al. Vitrectomy without gas tamponade for macular retinoschisis associated with normal-tension glaucoma. Ophthalmic Surg Lasers Imaging Retina. 2015;46(1):107-10.

57. Lai L, Javier T, Lee S, Gallemore RP. A Masquerade Case: Choroidal Hemangioma Misdiagnosed As Central Serous Retinopathy. Int Med Case Rep J. 2023;16:239-44.

58. Koundanya VV, Tripathy K. Syphilis Ocular Manifestations. StatPearls. Treasure Island (FL)2023.

59. Etheridge T, Bowen RC, Raven M, Snow KB, Urban AW, Chang JS. Ocular Syphilis: Clinical Manifestations and Treatment Course. WMJ. 2019;118(4):191-5.

60. Li SY, Birnbaum AD, Tessler HH, Goldstein DA. Posterior syphilitic uveitis: clinical characteristics, co-infection with HIV, response to treatment. Jpn J Ophthalmol. 2011;55(5):486-94.

61. Kuo A, Ziaee SM, Hosseini H, Voleti V, Schwartz SD, Kim NU, et al. The Great Imitator: Ocular Syphilis Presenting as Posterior Uveitis. Am J Case Rep. 2015;16:434-7.

62. Pichi F, Ciardella AP, Cunningham ET, Jr., Morara M, Veronese C, Jumper JM, et al. Spectral domain optical coherence tomography findings in patients with acute syphilitic posterior placoid chorioretinopathy. Retina. 2014;34(2):373-84.

63. Clement ME, Okeke NL, Hicks CB. Treatment of syphilis: a systematic review. JAMA. 2014;312(18):1905-17.

64. Jones RM, Brosseau LM. Aerosol transmission of infectious disease. J Occup Environ Med. 2015;57(5):501-8.

65. Sanches I, Carvalho A, Duarte R. WITHDRAWN: Who are the patients with extrapulmonary tuberculosis? Rev Port Pneumol. 2014.

66. Borde P, Priyanka, Kumar K, Takkar B, Sharma B. Pattern of uveitis in a tertiary eye care center of central India: Results of a prospective patient database over a period of two years. Indian J Ophthalmol. 2020;68(3):476-81.

67. Tabbara KF. Ocular tuberculosis: anterior segment. Int Ophthalmol Clin. 2005;45(2):57-69.

68. Gupta V, Gupta A, Rao NA. Intraocular tuberculosis--an update. Surv Ophthalmol. 2007;52(6):561-87.

69. Niederer RL, Sharief L, Tomkins-Netzer O, Lightman SL. Uveitis in Sarcoidosis - Clinical Features and Comparison with Other Non-infectious Uveitis. Ocul Immunol Inflamm. 2023;31(2):367-73.

70. Tejada P, Mendez MJ, Negreira S. Choroidal tubercles with tuberculous meningitis. Int Ophthalmol. 1994;18(2):115-8.

71. Sterling TR, Njie G, Zenner D, Cohn DL, Reves R, Ahmed A, et al. Guidelines for the treatment of latent tuberculosis infection: Recommendations from the National Tuberculosis Controllers Association and CDC, 2020. American Journal of Transplantation. 2020;20(4):1196-206.

72. Ziobrowski S, Zemburowa K, Laskownicka Z, Przybylkiewicz Z. [Uveitis in toxoplasmosis (author's transl)]. Klin Oczna. 1977;47(9):389-91.

73. Morais FB, Arantes T, Muccioli C. Current Practices in Ocular Toxoplasmosis: A Survey of Brazilian Uveitis Specialists. Ocul Immunol Inflamm. 2018;26(2):317-23.

74. Bornand JE, de Gottrau P. Uveitis: is ocular toxoplasmosis only a clinical diagnosis? Ophthalmologica. 1997;211(2):87-9.

75. Garweg JG, Pleyer U. Treatment Strategy in Human Ocular Toxoplasmosis: Why Antibiotics Have Failed. J Clin Med. 2021;10(5).

76. Pleyer U, Chee SP. Current aspects on the management of viral uveitis in immunocompetent individuals. Clin Ophthalmol. 2015;9:1017-28.

77. Wensing B, Relvas LM, Caspers LE, Valentincic NV, Stunf S, de Groot-Mijnes JD, et al. Comparison of rubella virus- and herpes virus-associated anterior uveitis: clinical manifestations and visual prognosis. Ophthalmology. 2011;118(10):1905-10.

78. Vassileva PI, Markov GT. Hypertensive Herpetic Uveitis - Diagnostic And Therapeutic Approach: A Case Series. Invest Ophth Vis Sci. 2012;53(14):2790-.

79. Sugita S, Takase H, Nakano S. Role of Recent PCR Tests for Infectious Ocular Diseases: From Laboratory-Based Studies to the Clinic. Int J Mol Sci. 2023;24(9).

80. Sungur GK, Hazirolan D, Yalvac IS, Ozer PA, Aslan BS, Duman S. Incidence and prognosis of ocular hypertension secondary to viral uveitis. International Ophthalmology. 2010;30(2):191-4.

81. Classification Criteria for Varicella Zoster Virus Anterior Uveitis. Am J Ophthalmol. 2021;228:165-73.

82. Zandi S, Bodaghi B, Garweg JG. Review for Disease of the Year: Treatment of Viral Anterior Uveitis: A Perspective. Ocular Immunology and Inflammation. 2018;26(7):1135-42.

83. Carmichael A. Cytomegalovirus and the eye. Eye (Lond). 2012;26(2):237-40.

84. Ye Z, Yang Y, Ke W, Li Y, Wang K, Chen M. Overview and update on cytomegalovirus-associated anterior uveitis and glaucoma. Front Public Health. 2023;11:1117412.

85. Okonkwo ON, Zeppieri M, Tripathy K. Posner-Schlossman Syndrome. StatPearls. Treasure Island (FL)2025.

86. Jabs DA. Ocular manifestations of HIV infection. Trans Am Ophthalmol Soc. 1995;93:623-83.

87. Kamkwalala AR, Garg A, Roy U, Matthews A, Castillo-Mancilla J, Lake JE, et al. Current Considerations for Clinical Management and Care of People with HIV: Findings from the 11th Annual International HIV and Aging Workshop. AIDS Res Hum Retroviruses. 2021;37(11):807-20.

88. Thorne JE, Shah KH, Brown DM, Holland GN, Jabs DA. Posterior intracorneal opacities in patients with HIV infection. Ocul Immunol Inflamm. 2005;13(1):25-31.

89. Elhamaky TR. Long-term efficacy of dexamethasone intravitreal implant in the treatment of Vogt-Koyanagi-Harada disease relapsing posterior uveitis. Indian J Ophthalmol. 2022;70(7):2465-70.

90. Jiang Y, Liu Q, Kong X, Zhao M, Liu Y, Gao P, et al. Role of HLA class I and II alleles in susceptibility to ankylosing spondylitis in Chinese Han. J Clin Lab Anal. 2023;37(17-18):e24964.

91. Martin TM, Rosenbaum JT. An update on the genetics of HLA B27-associated acute anterior uveitis. Ocul Immunol Inflamm. 2011;19(2):108-14.

92. Reveille JD, Zhou X, Lee M, Weisman MH, Yi L, Gensler LS, et al. HLA class I and II alleles in susceptibility to ankylosing spondylitis. Ann Rheum Dis. 2019;78(1):66-73.

93. Abd El Latif E, Abdelhalim AS. Clinical Profile of HLA-B27-Associated Uveitis in an Egyptian Cohort. Clin Ophthalmol. 2020;14:3821-8.

94. Rosenbaum JT. New developments in uveitis associated with HLA B27. Curr Opin Rheumatol. 2017;29(4):298-303.

95. Wakefield D, Clarke D, McCluskey P. Recent Developments in HLA B27 Anterior Uveitis. Front Immunol. 2020;11:608134.

96. Al-Ani HH, Sims JL, Niederer RL. Long term complications and vision loss in HLA-B27 uveitis. Eye (Lond). 2023;37(8):1673-7.

97. Thomas J, Kuthyar S, Shantha JG, Angeles-Han ST, Yeh S. Update on biologic therapies for juvenile idiopathic arthritis-associated uveitis. Ann Eye Sci. 2021;6.

98. Clarke SLN, Sen ES, Ramanan AV. Juvenile idiopathic arthritis-associated uveitis. Pediatric Rheumatology. 2016;14(1):27.

99. Heiligenhaus A, Minden K, Tappeiner C, Baus H, Bertram B, Deuter C, et al. Update of the evidence based, interdisciplinary guideline for anti-inflammatory treatment of uveitis associated with juvenile idiopathic arthritis. Semin Arthritis Rheum. 2019;49(1):43-55.

100. Heiligenhaus A, Deuter C. Uveitis in Juvenile Idiopathic Arthritis - Case Reports on Guideline-based Diagnostic Work-up and Treatment. Klin Monbl Augenheilkd. 2022;239(5):676-85.

101. Huang XF, Brown MA. Progress in the genetics of uveitis. Genes Immun. 2022;23(2):57-65.

102. Keino H, Okada AA. Behcet's disease: global epidemiology of an Old Silk Road disease. Br J Ophthalmol. 2007;91(12):1573-4.

103. Azizlerli G, Kose AA, Sarica R, Gul A, Tutkun IT, Kulac M, et al. Prevalence of Behcet's disease in Istanbul, Turkey. Int J Dermatol. 2003;42(10):803-6.

104. Tugal-Tutkun I, Cakar Ozdal P. Behcet's disease uveitis: is there a need for new emerging drugs? Expert Opin Emerg Drugs. 2020;25(4):531-47.

105. Tugal-Tutkun I, Onal S, Stanford M, Akman M, Twisk JWR, Boers M, et al. An Algorithm for the Diagnosis of Behcet Disease Uveitis in Adults. Ocul Immunol Inflamm. 2021;29(6):1154-63.

106. Tafti D, Ehsan M, Xixis KL. Multiple Sclerosis. StatPearls. Treasure Island (FL)2024.

107. Olsen TG, Frederiksen J. The association between multiple sclerosis and uveitis. Surv Ophthalmol. 2017;62(1):89-95.

108. Messenger W, Hildebrandt L, Mackensen F, Suhler E, Becker M, Rosenbaum JT. Characterisation of uveitis in association with multiple sclerosis. Br J Ophthalmol. 2015;99(2):205-9.

109. Ambrosius W, Michalak S, Kozubski W, Kalinowska A. Myelin Oligodendrocyte Glycoprotein Antibody-Associated Disease: Current Insights into the Disease Pathophysiology, Diagnosis and Management. Int J Mol Sci. 2020;22(1).

110. Ramanathan S, Fraser C, Curnow SR, Ghaly M, Leventer RJ, Lechner-Scott J, et al. Uveitis and optic perineuritis in the context of myelin oligodendrocyte glycoprotein antibody seropositivity. Eur J Neurol. 2019;26(8):1137-e75.

111. Ramanathan S, Reddel SW, Henderson A, Parratt JD, Barnett M, Gatt PN, et al. Antibodies to myelin oligodendrocyte glycoprotein in bilateral and recurrent optic neuritis. Neurol Neuroimmunol Neuroinflamm. 2014;1(4):e40.

112. Ramanathan S, Mohammad S, Tantsis E, Nguyen T, Merheb V, Fung V, et al. The clinical course, therapeutic responses, and outcomes in relapsing MOG antibody-associated demyelination. Mult Scler J. 2017;23(13):Np1-Np.

113. Rusescu B-V, Diederich NJ, Tsobo C, Marignier R, Kerschen P. MOG antibody-associated optic neuritis in the setting of acute CMV infection. Journal of the Neurological Sciences. 2017;382:44-6.

114. Jeyakumar N, Waller S, Mahant N, Ramanathan S, Henderson APD. A case of CSF anti-MOG antibody-positive papillitis with intermediate uveitis in the setting of acute neurosyphilis. Neuroimmunology Reports. 2021;1:100033.

115. Tavallali A, Yannuzzi LA. MEWDS, Common Cold of the Retina. J Ophthalmic Vis Res. 2017;12(2):132-4.

116. Cunningham ET, Levinson RD, Denniston AK, Brezin AP, Zierhut M. Birdshot Chorioretinopathy. Ocul Immunol Inflamm. 2017;25(5):589-93.

117. Thorne JE, Jabs DA, Peters GB, Hair D, Dunn JP, Kempen JH. Birdshot retinochoroidopathy: ocular complications and visual impairment. Am J Ophthalmol. 2005;140(1):45-51.

118. Brezin AP, Monnet D, Cohen JH, Levinson RD. HLA-A29 and birdshot chorioretinopathy. Ocul Immunol Inflamm. 2011;19(6):397-400.

119. Ma Y, Gal A, Koss M. Reprint of: The pathology of pulmonary sarcoidosis: update. Semin Diagn Pathol. 2018;35(5):324-33.

120. Pasadhika S, Rosenbaum JT. Ocular Sarcoidosis. Clin Chest Med. 2015;36(4):669-83.

121. Tugal-Tutkun I, Thorne JE, Smit DP. Sarcoid Uveitis. Ocul Immunol Inflamm. 2024;32(2):135-6.

122. Shen J, Su Z, Feng L. Sarcoid uveitis: A case report and systematic review of literature. Adv Ophthalmol Pract Res. 2022;2(2):100047.

123. Yang PZ, Tan HD, Ye Z, Yi SL. [Analysis on the clinical features of sarcoid uveitis]. Zhonghua Yan Ke Za Zhi. 2023;59(3):196-201.

124. Moorthy RS, Moorthy MS, Cunningham ET, Jr. Drug-induced uveitis. Curr Opin Ophthalmol. 2018;29(6):588-603.

125. London NJ, Garg SJ, Moorthy RS, Cunningham ET. Drug-induced uveitis. J Ophthalmic Inflamm Infect. 2013;3(1):43.

126. Patel KG, Hilton T, Choi RY, Abbey AM. Uveitis and Posterior Ophthalmic Manifestations Following the SARS-CoV-2 (COVID-19) Vaccine. Ocul Immunol Inflamm. 2022:1-7.

127. Nso N, Toz B, Ching TH, Kondaveeti R, Abrudescu A. Tattoo-Associated Sarcoidosis With Severe Uveitis Successfully Treated With Mycophenolate Mofetil: A Report of Two Cases. Cureus. 2021;13(8):e17197.

128. Weng CY, Albini TA. Syphilitic posterior uveitis. JAMA Ophthalmol. 2015;133(4):e144630.

129. Van der Lelij A, Ooijman FM, Kijlstra A, Rothova A. Anterior uveitis with sectoral iris atrophy in the absence of keratitis: a distinct clinical entity among herpetic eye diseases. Ophthalmology. 2000;107(6):1164-70.

130. Wakefield D, Yates W, Amjadi S, McCluskey P. HLA-B27 Anterior Uveitis: Immunology and Immunopathology. Ocul Immunol Inflamm. 2016;24(4):450-9.

131. International Team for the Revision of the International Criteria for Behcet's D. The International Criteria for Behcet's Disease (ICBD): a collaborative study of 27 countries on the sensitivity and specificity of the new criteria. J Eur Acad Dermatol Venereol. 2014;28(3):338-47.

132. Sadda SR. Charting a research agenda for macular degeneration: Reviews from the Ryan Initiative for Macular Research. Exp Eye Res. 2023;226:109348.

133. Tsirouki T, Dastiridou A, Symeonidis C, Tounakaki O, Brazitikou I, Kalogeropoulos C, et al. A Focus on the Epidemiology of Uveitis. Ocul Immunol Inflamm. 2018;26(1):2-16.

134. McCluskey PJ, Towler HM, Lightman S. Management of chronic uveitis. BMJ. 2000;320(7234):555-8.

135. Prieto-Del-Cura M, Gonzalez-Guijarro JJ. Risk factors for ocular complications in adult patients with uveitis. Eur J Ophthalmol. 2020;30(6):1381-9.

136. Choi RY, Rivera-Grana E, Rosenbaum JT. Reclassifying Idiopathic Uveitis: Lessons From a Tertiary Uveitis Center. Am J Ophthalmol. 2019;198:193-9.

n

137. Nakayama LF, Ribeiro LZ, Dychiao RG, Zamora YF, Regatieri CVS, Celi LA, et al. Artificial intelligence in uveitis: A comprehensive review. Surv Ophthalmol. 2023;68(4):669-77.

138. Rosenbaum JT, Harrington CA, Searles RP, Fei SS, Zaki A, Arepalli S, et al. Revising the Diagnosis of Idiopathic Uveitis by Peripheral Blood Transcriptomics. Am J Ophthalmol. 2021;222:15-23.

139. Sandhu HS, Kaplan HJ. Chapter 3 - Approach to Therapy. In: Sandhu HS, Kaplan HJ, editors. Clinical Cases in Uveitis. Philadelphia: Elsevier; 2021. p. 9-19.

140. Munoz-Fernandez S, Martin-Mola E. Uveitis. Best Pract Res Clin Rheumatol. 2006;20(3):487-505.

141. Yanoff M, Sassani JW. 3 - Nongranulomatous Inflammation: Uveitis, Endophthalmitis, Panophthalmitis, and Sequelae. In: Yanoff M, Sassani JW, editors. Ocular Pathology (Eighth Edition). London: Elsevier; 2020. p. 62-76.e2.

142. Pichi F, Neri P. Complications in Uveitis. 1st 2020. ed. Cham: Springer International Publishing; 2020.

143. Forrester JV, Xu H, Lambe T, Cornall R. Immune privilege or privileged immunity? Mucosal Immunol. 2008;1(5):372-81.

144. Medawar PB. Immunity to homologous grafted skin; the fate of skin homografts transplanted to the brain, to subcutaneous tissue, and to the anterior chamber of the eye. Br J Exp Pathol. 1948;29(1):58-69.

145. Hori J, Niederkorn JY. Immunogenicity and immune privilege of corneal allografts. Chem Immunol Allergy. 2007;92:290-9.

146. Zhou R, Caspi RR. Ocular immune privilege. F1000 Biol Rep. 2010;2.

o

147. O'Leary F, Campbell M. The blood–retina barrier in health and disease. The FEBS Journal. 2023;290(4):878-91.

148. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. Nature Reviews Molecular Cell Biology. 2013;14(2):98-112.

149. Stein-Streilein J. Immune regulation and the eye. Trends Immunol. 2008;29(11):548-54.

150. Balamurugan S, Das D, Hasanreisoglu M, Toy BC, Akhter M, Anuradha VK, et al. Interleukins and cytokine biomarkers in uveitis. Indian J Ophthalmol. 2020;68(9):1750-63.

151. Griffith TS, Ferguson TA. The role of FasL-induced apoptosis in immune privilege. Immunol Today. 1997;18(5):240-4.

152. Griffith TS, Brunner T, Fletcher SM, Green DR, Ferguson TA. Fas ligand-induced apoptosis as a mechanism of immune privilege. Science. 1995;270(5239):1189-92.

153. Monie TP. Section 1 - A Snapshot of the Innate Immune System. In: Monie TP, editor. The Innate Immune System: Academic Press; 2017. p. 1-40.

154. Yoshimura T, Matsushima K, Oppenheim JJ, Leonard EJ. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). 1987. J Immunol. 2005;175(9):5569-74.

155. Zhang JM, An J. Cytokines, inflammation, and pain. Int Anesthesiol Clin. 2007;45(2):27-37.

156. Janeway's Textbook of immunology (2016).Chapter 4 page 192.

157. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. Nat Rev Immunol. 2012;12(3):180-90.

158. Bradley JR. TNF-mediated inflammatory disease. J Pathol. 2008;214(2):149-60.

159. Dinarello CA. A clinical perspective of IL-1β as the gatekeeper of inflammation. Eur J Immunol. 2011;41(5):1203-17.

160. Kishimoto T. Interleukin-6: from basic science to medicine--40 years in immunology. Annu Rev Immunol. 2005;23:1-21.

161. Mosser DM, Zhang X. Interleukin-10: new perspectives on an old cytokine. Immunol Rev. 2008;226:205-18.

162. Travis MA, Sheppard D. TGF-β activation and function in immunity. Annu Rev Immunol. 2014;32:51-82.

163. Lee J, Lozano-Ruiz B, Yang FM, Fan DD, Shen L, Gonzalez-Navajas JM. The Multifaceted Role of Th1, Th9, and Th17 Cells in Immune Checkpoint Inhibition Therapy. Front Immunol. 2021;12:625667.

164. Kothur K, Wienholt L, Brilot F, Dale RC. CSF cytokines/chemokines as biomarkers in neuroinflammatory CNS disorders: A systematic review. Cytokine. 2016;77:227-37.

165. Kothur K, Gill D, Wong M, Mohammad SS, Bandodkar S, Arbunckle S, et al. Cerebrospinal fluid cyto-/chemokine profile during acute herpes simplex virus induced anti-N-methyl-d-aspartate receptor encephalitis and in chronic neurological sequelae. Dev Med Child Neurol. 2017;59(8):806-14.

166. Kothur K, Wienholt L, Mohammad SS, Tantsis EM, Pillai S, Britton PN, et al. Utility of CSF Cytokine/Chemokines as Markers of Active Intrathecal Inflammation: Comparison of Demyelinating, Anti-NMDAR and Enteroviral Encephalitis. PLoS One. 2016;11(8):e0161656.

167. Liu C, Chu D, Kalantar-Zadeh K, George J, Young HA, Liu G. Cytokines: From Clinical Significance to Quantification. Advanced Science. 2021;8(15):2004433.

168. Leung TG, Thorne JE. Emerging drugs for the treatment of uveitis. Expert Opin Emerg Drugs. 2013;18(4):513-21.

169. Ferreira LB, Williams KA, Best G, Haydinger CD, Smith JR. Inflammatory cytokines as mediators of retinal endothelial barrier dysfunction in non-infectious uveitis. Clin Transl Immunology. 2023;12(12):e1479.

170. Sauer A, Villard O, Creuzot-Garcher C, Chiquet C, Berrod JP, Speeg-Schatz C, et al. Intraocular levels of interleukin 17A (IL-17A) and IL-10 as respective determinant markers of toxoplasmosis and viral uveitis. Clin Vaccine Immunol. 2015;22(1):72-8.

171. Errera MH, Pratas A, Fisson S, Manicom T, Boubaya M, Sedira N, et al. Cytokines, chemokines and growth factors profile in human aqueous humor in idiopathic uveitis. PLoS One. 2022;17(1):e0254972.

172. Weinstein JE, Pepple KL. Cytokines in uveitis. Current Opinion in Ophthalmology. 2018;29(3):267-74.

173. Xu J, Qin Y, Chang R, Tan H, Wang Q, Su G, et al. Aqueous cytokine levels in four common uveitis entities. Int Immunopharmacol. 2020;78:106021.

174. Ang M, Cheung G, Vania M, Chen J, Yang H, Li J, et al. Aqueous cytokine and chemokine analysis in uveitis associated with tuberculosis. Mol Vis. 2012;18:565-73.

175. Horai R, Caspi RR. Cytokines in autoimmune uveitis. J Interferon Cytokine Res. 2011;31(10):733-44.

176. Azzam D, Ronquillo Y. Snellen Chart. StatPearls. Treasure Island (FL)2023.

177. Perera C, Chakrabarti R, Islam FMA, Crowston J. The Eye Phone Study: reliability and accuracy of assessing Snellen visual acuity using smartphone technology. Eye. 2015;29(7):888-94.

r

178. Kumar H, Thulasidas M. Comparison of Perimetric Outcomes from Melbourne Rapid Fields Tablet Perimeter Software and Humphrey Field Analyzer in Glaucoma Patients. Journal of Ophthalmology. 2020;2020(1):8384509.

179. Ruia S, Tripathy K. Humphrey Visual Field. StatPearls. Treasure Island (FL)2024.

180. Invernizzi A, Cozzi M, Staurenghi G. Optical coherence tomography and optical coherence tomography angiography in uveitis: A review. Clinical & Experimental Ophthalmology. 2019;47(3):357-71.

181. Marmoy OR, Henderson RH, Ooi K. Recommended protocol for performing oral fundus fluorescein angiography (FFA) in children. Eye. 2022;36(1):234-6.

182. Gelişken F. Indocyanine Green Angiography. Turk J Ophthalmol. 2024;54(1):38-45.

183. Holladay JT. Visual acuity measurements. J Cataract Refract Surg. 2004;30(2):287-90.

184. Guedes MCE, Martins CG, Arroz MJ, Angelo-Dias M, Borrego LM, Proenca RD. Cytokine profiles in the peripheral blood and aqueous humor of patients with herpetic uveitis. J Ophthalmic Inflamm Infect. 2020;10(1):37.

185. Meng T, Nie L, Wang Y. Role of CD4(+) T cell-derived cytokines in the pathogenesis of uveitis. Clin Exp Med. 2025;25(1):49.

186. Jenh CH, Cox MA, Hipkin W, Lu T, Pugliese-Sivo C, Gonsiorek W, et al. Human B cell-attracting chemokine 1 (BCA-1; CXCL13) is an agonist for the human CXCR3 receptor. Cytokine. 2001;15(3):113-21.