

CHAPTER 13

Summary, conclusions and considerations

SUMMARY

The fast identification of infectious uveitis entities is of crucial importance since their treatment and visual prognosis differ entirely from noninfectious intraocular inflammations. In Europe, the infections are thought to cause approximately 20-25% of cases, whereas about 30% is associated with a noninfectious disease. The remainder is of (yet) unknown cause and might be associated with until now undiagnosed infections. Analysis of peripheral blood does not provide conclusive evidence for the diagnosis of intraocular infections. It is therefore imperative to establish the causative agent by the examination of intraocular fluid. The aim of this thesis was to discover novel causes of infectious uveitis by implementing new diagnostic assays on ocular fluids and gain more insight in the (immuno) pathogenesis of infectious uveitis.

Chapter 1 is a general introduction and an up-to-date review of the literature on ocular fluid analysis and the etiology of infectious uveitis. Intraocular fluid analyses by means of PCR and the detection of intraocular antibody production by Goldmann-Witmer coefficient (GWC) determination are described and discussed. Furthermore, known infectious causes of uveitis and their corresponding diagnostic assays are described. Finally, possible novel infectious entities of uveitis together with their diagnostic means are addressed, including Rubella virus, Parvovirus B19, Human parechovirus and Human herpesvirus 6.

In **Chapter 2** we investigated whether Rubella virus is associated with Fuchs heterochromic uveitis syndrome (FHUS) by analyzing intraocular antibody production (GWC determination) against Rubella virus in 14 patients with clinically established FHUS, in 13 control patients with herpetic anterior uveitis (AU) and 19 control patients with ocular toxoplasmosis (OT). Intraocular antibody production against Rubella virus was found in 13/14 (93%) patients. These patients were negative for Herpes simplex virus (HSV), Varicella zoster virus (VZV) and *Toxoplasma gondii*. None of the control patients showed intraocular antibody production against Rubella virus. We concluded that Rubella virus, and not HSV, VZV and *T. gondii*, is associated with FHUS.

In **Chapter 3** we investigated the clinical profile of 30 patients with chronic anterior uveitis (AU) and a positive intraocular analysis for Rubella virus, and assessed similarities to FHUS. Clinical records were compared with the profiles of 13 patients with chronic AU of undetermined origin. Patients with Rubella virus-associated uveitis appeared to be younger at the time of initial ophthalmologic presentation and presented more frequently with unilateral ocular involvement, keratic precipitates, iris atrophy and/or heterochromia, vitreous opacities and cataract. Also, the combination of main FHUS symptoms (keratic precipitates, the absence of posterior synechiae, cataract and vitreous opacities) occurred more often in the Rubella virus-positive group. We concluded that Rubella virus causes a distinct clinical spectrum of ocular symptoms similar to the FHUS and suggest that the virus is implicated in the pathogenesis of FHUS.

Patients with FHUS have a high prevalence of (chorio)retinal lesions, reminiscent of toxoplasmic scars. In **Chapter 4** we investigated whether the clinical appearance of these focal (chorio)retinal lesions differed between patients with intraocular Rubella virus infection and patients with intraocular *T. gondii* infection. Photographic and angiographic records were masked for identification and for infectious agent and evaluated by four specialists in the field of OT. No differences were observed between the retinal lesions in Rubella virus-positive and *T. gondii*-positive patients. By at least three out of four experts, retinal lesions were considered consistent with the diagnosis of OT in 55% of Rubella virus-positive patients and in 88% of *T. gondii*-positive patients. The retinal lesions in *T. gondii*-positive patients were more frequently considered “consistent with the diagnosis of ocular toxoplasmosis” by two experts. There was a ‘substantial agreement’ between the four experts. We concluded that clinical features of chorioretinal lesions in patients with intraocular Rubella virus infection were not distinct from those in patients with OT, indicating that the etiological diagnosis of these lesions cannot be made on clinical grounds solely.

We found a high seroprevalence for *T. gondii* in our Rubella virus-associated FHUS (RV-FHUS) patients. However, patients with FHUS have so far never presented with active retinal lesions and other symptoms compatible with OT. Moreover, we report on a *Toxoplasma*-seronegative case of RV-FHUS who had chorioretinal scars as well. Apparently, further investigations are required to elucidate this cause of retinal lesions in FHUS.

In **Chapter 5** we investigated the contribution of *Toxocara canis* to posterior uveitis of undetermined origin by means of GWC determination in 37 adult patients and 12 children. None of 37 adults had a positive GWC, whereas three of twelve children demonstrated intraocular antibody production against *Toxocara canis*. All three had very low or undetected serum titers, and their intraocular antibody titers exceeded those in the peripheral blood. One child had vitritis, one presented with a low-grade uveitis and a peripheral retinal lesion and the third patient had posterior uveitis with a chorioretinal scar. We concluded that ocular toxocariasis is mainly a pediatric disease. Our findings underline that serological screening alone is not informative for the diagnosis of ocular toxocariasis and GWC analysis can be of value when diagnosing (young) patients with posterior focal lesions or vitritis of unknown etiology.

In **Chapter 6** we reported on an adult patient who presented with a decrease in visual acuity of his right eye, cells and mild opacities in the vitreous, and a white retinal infiltrate in the posterior pole. Aqueous analysis revealed intraocular antibody production against *Toxocara canis*, despite negative serology. After treatment with antihelminthics the retinal infiltrate decreased in size. Although ocular toxocariasis is mainly a pediatric disease (Chapter 5), one should be aware that it may also occur in adults. It is important to perform ocular fluid analysis and determine the GWC, even when *Toxocara* serology is negative, as early diagnosis and intervention provide better outcomes.

In **Chapter 7** we analyzed 139 ocular fluids samples of patients suspected of infectious uveitis, but negative for the most common inciting agents HSV, VZV, Cytomegalovirus (CMV) and *T. gondii*, for 18 viruses and three bacteria by PCR. Positive PCR results were found in seven patients: one was positive for Epstein-Barr virus (EBV), one for Rubella virus, one for Human herpesvirus 6 and four patients were positive for Human parechovirus. The latter observation is particularly interesting, as Human parechovirus infections mainly occur during childhood, whereas here all four patients were adults. One patient was immunocompromised and was suspected of ocular syphilis. The other three patients all had AU of unknown origin associated with corneal involvement and cells in the anterior chamber. We concluded that Human parechovirus may be a novel cause of infectious uveitis.

We hypothesized that like *T. gondii*, HSV and Rubella virus, other childhood infections might also be able to incite uveitis. To this end we determined whether Parvovirus B19, Mumps virus and Measles virus are associated with AU (**Chapter 8**) or with intermediate uveitis, neuroretinitis and focal chorioretinitis of non-toxoplasmic origin (**Chapter 9**) by GWC analysis. In addition, CMV as a cause of AU was investigated (**Chapter 8**). We identified two patients with unexplained AU and positive GWCs against CMV and one AU patient who was positive for Parvovirus B19. Intraocular antibody production against Mumps- or Measles virus was not detected. None of the patients with intermediate or posterior uveitis had a positive GWC for one of the investigated pathogens. We concluded that CMV and Parvovirus B19 may be associated with AU and suggested AH analysis for these pathogens in patients with unexplained AU. We found no laboratory evidence for the involvement of Parvovirus B19, Mumps virus and Measles virus in the pathogenesis of intermediate uveitis, neuroretinitis and focal chorioretinitis.

In order to identify cytokines and chemokines that may play a role in the immunopathogenesis of three important types of infectious uveitis, paired serum and aqueous humor samples were analyzed by multiplex immunoassay in 18 patients with RV-FHUS of 20 patients with OT, and of 19 patients with acute retinal necrosis (ARN) (**Chapter 10**). The results showed that RV-FHUS and OT revealed similar patterns of mediator production, which were different from ARN. ARN samples had higher overall cytokine levels than RV-FHUS and OT samples, however, IL-12 levels were significantly higher in RV-FHUS and OT patients, compared to ARN patients and the controls. On the other hand, IL-10 and IL-18 levels were significantly higher in ARN compared to RV-FHUS, OT and the controls. IFN γ levels were elevated in ARN samples. No correlation was found between cytokine levels and the interval between the onset of symptoms and the time of sampling. Also no correlation could be found between the use of corticosteroids and cytokine levels. We concluded that the differences in immune mediator expression between RV-FHUS and OT on the one hand and ARN on the other, may be related to clinical disease activity and severity. No explicit T helper (Th) pathway could be identified for either uveitis entity. Apparently, both Th1 and Th2 associated mediators are involved.

In **Chapter 11** we analyzed intraocular and serum levels of vascular endothelial growth factor (VEGF) in 17 patients with ARN and 16 patients with OT by immunoassay to determine whether the size and severity of retinal inflammation are related to intraocular VEGF formation. We found that intraocular VEGF levels in patients with ARN were higher than in OT patients while their serum VEGF levels did not differ. Intraocular VEGF levels exceeded the serum levels in 47% of patients with ARN compared to 6% in OT. Furthermore, we found that the patients with intraocular VEGF levels exceeding those in serum had a more extensive retinitis and lower visual acuity at the three months follow-up ($P < 0.001$ and $P = 0.031$ respectively). We concluded that high intraocular VEGF levels in patients with ARN are associated with extensive retinitis and poor visual prognosis. High local VEGF production in ARN might be of importance for future treatment of patients with this devastating ocular disease.

In **Chapter 12** we described a pilot study in which we attempt to detect specific protein profiles by Surface enhanced laser desorption/ionization time-of-flight (Seldi-tof) in the vitreous of five patients with acute postoperative endophthalmitis, three of whom had a positive and two had a negative culture for staphylococci. As controls we included three vitreous fluid samples of patients with a macular hole, without any inflammation. Our data showed that the patients with endophthalmitis had similar protein profiles, clearly different from those of the control patients.

In addition, we analyzed paired aqueous humor and serum samples of patients with intraocular antibody production against HSV (n=10) and *T. gondii* (n=8), and control patients with age-related cataract (n=7). These experiments revealed a peak in the aqueous humor that appeared to be specific for *T. gondii*. These preliminary data indicate that ocular fluids are suitable for Seldi-tof analysis and that this technique might be of value for detection of specific intraocular biomarkers. Further investigations are required to determine the relevance of our observations.

CONCLUSIONS AND CONSIDERATIONS

In this thesis we report on an association of Rubella virus with Fuchs Heterochromic Uveitis Syndrome (FHUS). The majority of our patients with clinically established FHUS had intraocular antibody production against Rubella virus. Furthermore, patients with Rubella virus-associated uveitis had a distinct clinical spectrum of ocular symptoms similar to patients with FHUS. However, the four classical clinical criteria of FHUS (characteristic keratic precipitates, iris atrophy and/or heterochromia, absence of posterior synechiae and cataract) were also observed in patients who had negative results for intraocular antibody production against Rubella virus, which suggests that other causes of FHUS might exist. Indeed, recently CMV was associated with FHUS. Therefore it is probable that FHUS is a clinical syndrome which might have multiple causes and additional pathogens associated with FHUS might be identified in the future.

In the past, many different names have been used to describe a Fuchs Heterochromic Uveitis Syndrome (FHUS), including Fuchs' heterochromic (irido) cyclitis, Fuchs' anterior uveitis and Fuchs' heterochromic uveitis, which explains the different names in the various chapters of this thesis. Recently however, it has been decided by the International Uveitis Study Group to address this clinical syndrome as 'Fuchs Heterochromic Uveitis Syndrome'. When a specific etiology is identified, it should be referred to as for example Rubella virus-associated Fuchs Heterochromic Uveitis or CMV-associated Fuchs Heterochromic Uveitis.

The presence of toxoplasmosis-like chorioretinal lesions in patients with Rubella virus-associated Fuchs uveitis syndrome (RV-FHUS) is intriguing. Our study clearly demonstrated that the etiological diagnosis cannot be made on clinical grounds solely and that aqueous analysis is required to establish the definitive diagnosis. The question still remains which pathogen causes the chorioretinal scars in patients with RV-FHUS.

In this thesis, we analyzed ocular fluids samples from patients with undetermined uveitis for a variety of pathogens. We found positive results for Human parechovirus, Human herpesvirus 6, for Parvovirus B19 and CMV and conclude that these pathogens might be associated with infectious uveitis. Further investigation into the role of Human parechovirus and Human herpesvirus 6 in

ocular disease has to be performed to determine whether these viruses are true causes of infectious uveitis. Furthermore, since the number of patients tested for CMV, Parvovirus B19, Mumps and Measles virus was rather small, we cannot exclude the involvement of these viruses in pathogenesis of uveitis. It would be interesting to analyze a large number of patients with uveitis of unknown etiology for these viruses and include PCR assays in these studies.

Our studies revealed that GWC analysis for ocular toxocariasis can be of value when diagnosing patients with posterior focal lesions or vitritis of unknown etiology, especially in children. However, it should be emphasized that ocular toxocariasis may also occur in adults. It is important to realize that intraocular fluid analysis is essential for the diagnosis as serological screening against *Toxocara canis* is not informative and serology can be false-negative. In future, it would be of value to investigate if other nematodes, such as *Ascaris lumbricoides*, play a role in the pathogenesis of infectious uveitis.

In a comprehensive study into the role of cytokines and chemokines in infectious uveitis, we observed that ocular fluids of patients with RV-FHUS and OT reveal a similar pattern of cytokine and chemokine production distinct from that of ARN. Ocular fluids of patients with ARN exhibited higher levels of mediators, which might correlate with higher clinical disease activity and severity. Furthermore, levels of vascular endothelial growth factor (VEGF), which plays a crucial role in the intraocular ischemic processes and subsequent development of neovascularizations, appear to be significantly higher in the ocular fluids of patients with ARN compared to OT. Analysis of a larger number of patients and a comparison with noninfectious uveitis, preferably in homogenous groups of patients, might give further insight in the immunopathogenesis of ARN, RV-FHUS and OT. Also, it would be of value to determine whether anti-VEGF treatment would contribute to a better outcome and prognosis of ARN.

The new findings described in this thesis are of value for the diagnosis of infectious uveitis and have expanded the spectrum of causative agents involved in infectious uveitis. Analysis of intraocular fluids, especially the combination of detection of intraocular antibody production and PCR, is of value in diagnosing

patients with infectious uveitis and allows the early discrimination between infectious and noninfectious uveitis entities, which is important for prognosis and treatment of the patients. The current improvement of the diagnostic procedures and implementation of new diagnostic assays will enhance the detection of yet unknown infectious causes of uveitis. Moreover, together with a better understanding of the (immuno)pathogenesis of infectious uveitis, the expansion of the diagnostic repertoire will further improve our knowledge of potentially blinding but frequently treatable intraocular infections.