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

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ABSTRACT

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

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

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

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

Keywords: Aqueous fluid analysis, clinical picture, Epstein–Barr virus, uveitis, visual prognosis

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

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

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

METHODS

Patients and Data Collection

All patients who underwent diagnostic aqueous fluid analysis between January 2010 and October 2016 at the Ophthalmology department of the Erasmus Medical

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Center (EMC, Rotterdam, the Netherlands) were included in this retrospective cohort study, which was approved by the Medical Ethics Committee and adheres to the Tenets of the Declaration of Helsinki (MEC-2012–016). We reviewed the medical records of all patients who had positive results in PCR or GWC for EBV.[15]

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

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

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

Sample Collection and Processing

The ocular fluid samples were stored at −80°C and serum samples at +4°C until processing for laboratory analysis.

Determination of Intraocular Antibody Production: Specific immunoglobulin G (IgG) titers against RV, HSV, VZV, CMV, and EBV in serum and aqueous humor were determined with the Euroimmun (Luebeck, Germany) indirect immunofluorescence test kit. The immunofluorescence assays (IFAs) are based on biochips, which were coated with the virus specific-infected cells. Serial tenfold dilutions (1:10 to 1:5120) were prepared in sample buffer (Euroimmun). Samples were applied to the reaction fields of a reagent tray. After incubation for 30 min, slides were rinsed and immersed with phosphate-buffered saline (PBS). For detection of bound antibodies, slides were placed on reagent trays prepared with fluorescein conjugated antihuman immunoglobulin of the IgG class. Following a 30-min incubation, slides were washed as described above, embedded with mounting medium, cover slipped and evaluated by fluorescence microscopy.

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

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

Twenty µL extracted RNA was amplified in 50-µL final volume, containing 12.5-µL 4 × TaqMan Fast Virus 1-Step Master Mix (including (1 U/µL) uracil-N-glycosylase, Life Technologies, Nieuwerkerk a/d IJssel, the Netherlands), and 1 µL of a primers and probe mixture. For DNA viruses 5 µL of trifluoroacetic acid (TFA) and 0.4 µL of primers and probe mixture was amplified in a 20-µL final volume. For CMV a dual target PCR was used.[17,18] For EBV, HSV1 and 2 and VZV primers were adapted from our earlier published procedure using real-time technique. Rubella RNA was amplified using forward primer (5′-cgtccagcaccctcacaag-3′), reverse primer (5′-cggagagttgccagacggt-3′) and probe (FAM-cgtccgggtcagttccatacagaga-BHQ-1). The RT-PCR temperature profile was 5 min at 50°C, 20 sec at 95°C, 45 cycles of 3 s at 95°C and 30 sec at 60°C. Amplification was performed in an LC480 II(Roche Applied Science, Almere, the Netherlands) using the Fit Point analysis module. Quality assurance was performed using QCtoday software. The criterion for a successful RT-PCR run was that cycle threshold (Ct) values of both internal control and positive RT-PCR control should be within 3 × standard deviation (SD) of the mean.

RESULTS

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

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

	Positive PCR in tested patients	Positive GWC (≥3) in tested patients	Positive GWC (≥3 but <10) in tested patients	Positive (GWC ≥10) in tested patients	Positive PCR and GWC (≥3.0) in tested patients
Herpes simplex virus	10/271 (4%)*	1/257 (<1%)	1/1 (100%)	0	0
Varicella zoster virus	9/271 (3%)	19/258 (7%)	11/19 (58%)	8/19 (42%)	7/245 (3%)
Cytomegalovirus	12/248 (5%)	13/252 (4%)	10/13 (77%)	3/13 (23%)	2/227 (1%)
Epstein–Barr virus	3/201 (1%)	22/245 (9%)	20/22 (91%)	2/22 (9%)	0
Rubella virus	9/183 (5%)	29/192 (15%)	8/29 (28%)	21/29 (72%)	7/167 (4%)
Toxoplasma gondii	6/120 (5%)	12/106 (11%)	6/12 (50%)	6/12 (50%)	3/101 (3%)

PCR, polymerase chain reaction; GWC, Goldmann–Witmer coefficient.
**9/271 (3%) Herpes simplex virus type 1 and 1/272 (<1%) Herpes simplex virus Type 2.*

Both assays were simultaneously performed in 184/297; 62% patients.

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

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

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

TABLE 2. Characteristics of patients tested for Epstein–Barr virus in intraocular fluids.

	Total	PCR and/or GWC negative	PCR positive for EBV	GWC positive for EBV
Number	297	272/297 (92%)	3/297 (1%)	22/297 (7%)
Age at onset uveitis *(mean years ± SD)*	46.4 (±18.8)	46.9 (±18.8)	50.7 (±11.8)	40.0 (±17.5)
Gender				
Male	121/297 (41%)	112/272 (41%)	1/3 (33%)	8/22 (36%)
Female	176/297 (59%)	160/272 (59%)	2/3 (67%)	14/22 (64%)
Anatomical localization				
Anterior	97/297 (33%)	93/272 (34%)	0	4/22 (18%)
Intermediate	26/297 (9%)	26/272 (10%)	0	0/22 (5%)
Posterior	84/297 (28%)	82/272 (30%)	1/3 (33%)	1/22 (5%)
Panuveitis	82/297 (28%)	65/272 (24%)	2/3 (67%)	15/22 (68%)
Scleritis	8/297 (3%)	6/272 (2%)	0	2/22 (9%)

EBV, Epstein–Barr virus; PCR, polymerase chain reaction; GWC, Goldmann–Witmer coefficient; SD, standard deviation.

TABLE 3. Ophthalmologic characteristics of patients positive in polymerase chain reaction for Epstein–Barr virus.

	EBV PCR	EBV GWC	Other PCR+	Other GWC+	IS at moment of aqueous fluid tap	Laterality	Localization	KPs	AU	Iris syn	Vitritis	Fundus	Alternative diagnosis
Patient 1	+*	–	–	–	–	2	Panuveitis	+	+	+	+	MFC	None
Patient 2	+*	–	T. Gondii	–	–	1	Posterior	–	–	–	+	Focal retinal lesion	Toxoplasmosis
Patient 3	+*	–	HIV-2	5.15 (CMV)	HIV+	1	Panuveitis	–	+	+	+	–	HIV-associated uveitis†

EBV, Epstein–Barr virus; GWC, Goldman–Wittmer coefficient; IS, immunosuppression; KP, keratic precipitates; AU, anterior uveitis; syn, synechia; MFC, multifocal chorioretinitis; CMV, cytomegalovirus; HIV, human immunodeficiency virus.

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

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

In total, 14 patients had laboratory results indicating only EBV infection (either 1. positive EBV-PCR with negative results for PCR and/or GWC for other viruses or 2. a negative EBV-PCR but GWC positive for EBV and in cases with multiple positive coefficients, GWC for EBV had the highest value). Out of these, eight (57%) patients had another explanation for their uveitis. The GWC values of these patients were between 3 and 10 in six of eight patients. No alternative explanation for uveitis was found in six (43%) patients. Three of these patients exhibited solely anterior chamber inflammation mostly with small KPs and marked involvement of the vitreous. Their vitritis was severe (requiring pars plana vitrectomy in two) but had no documented inflammatory involvement of the retina and/or choroid.[16] The remaining three patients had solely anterior chamber inflammation without vitreous and/or choroido-retinal involvement. All of these six patients had a chronic recurrent course of inflammations and good visual prognosis (all affected eyes had visual acuity at least of 20/20 at last follow-up. Only one of these six patients required systemic immunosuppressive treatment. The inflammation was bilateral in four of six patients and no other common characteristics were found. None of these six patients had aqueous fluid tap performed within 3 months after uveitis onset and their serum IgG levels for EBV were diverse (supplementary Table).

None of the 25 patients PCR and/or GWC positive patients for EBV had lymphoma at the onset of uveitis and/or was diagnosed with (intraocular) lymphoma during follow-up.

DISCUSSION

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

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

TABLE 4. Ophthalmologic characteristics of patients positive for Goldman–Wittmer coefficient of Epstein–Barr virus.

	EBV PCR	EBV GWC	Other PCR+	Other GWC +	IS at moment of aqueous fluid tap	Laterality	Localization	KPs	AU	Iris syn	Vitritis	Fundus	Alternative diagnosis
Patient 1	–	3.44	–	–	–	1	Panuveitis	–	+	–	+	–	None
Patient 2	–	5.50	–	–	–	1	Anterior	–	+	–	–	–	None
Patient 3	–	8.25	–	–	–	2	Panuveitis	+	+	+	+	–	None
Patient 4	–	9.31	–	4.66 (VZV)	–	2	Anterior	–	+	–	–	–	None
Patient 5	–	5.31	–	–	–	2	Anterior	+	+	–	–	–	None
Patient 6	–	41.39	–	–	HIV+	1	Panuveitis	–	–	–	+	POL Vasculitis	Sarcoidosis
Patient 7	–	3.74	–	–	–	2	Panuveitis	–	+	+	+	Granuloma's	Sarcoidosis
Patient 8	–	7.86	–	–	–	2	Panuveitis	+	+	–*	+	POL	Sarcoidosis
Patient 9	–	9.29	–	–	Adalimumab + prednisolone	2	Panuveitis	+	+	–	+	Peripheral retinal scar	Sarcoidosis
Patient 10	–	11.70	–	–	–	1	Panuveitis	–	+	+	–	POL	Sarcoidosis
Patient 11	–	3.48	–	–	–	2	Panuveitis	+	+	–	+	–	LTBI-associated uveitis
Patient 12	–	4.23	–	–	Adalimumab + methotrexate	2	Panuveitis	–	+	+	+	Vasculitis	HLA-B27+, psoritic arthritis, associated uveitis
Patient 13	–	4.29	–	–	–	2	Panuveitis	+	+	+	+	–	Multiple Sclerosis
Patient 14	0	3.63	+ (CMV)	–	HIV+	1	Panuveitis	+	+	–	+	Occlusive vasculitis	CMV-associated uveitis
Patient 15	–	3.17	+ (Toxoplasmosis)	–	HIV+	1	Panuveitis	+	+	+	+	Retinal detachment	Toxoplasmosis
Patient 16†	–	4.35	+ (RV)	–	–	1	Panuveitis	+	+	–	+	–	RV-associated uveitis
Patient 17	–	4.07	+ (CMV)	260.5 (CMV)	Post kidney-transplantion	2	Posterior	–	–	–	+	CMV-retinitis	CMV-associated uveitis
Patient 18	–	3.43	–	3.43 (HSV)	–	2	Panuveitis	+	+	+	+	–	Multiple sclerosis
Patient 19	0	7.88	–	7.88 (VZV)	–	1	Scleritis	–	–	–	–	–	Varicella Zoster
Patient 20	–	4.63	–	4.63 (VZV)	–	2	Anterior	+	+	–	–	–	Kikuchi's disease
Patient 21	–	5.06	–	5.06 (VZV)	–	1	Scleritis	+	–	–	–	–	Unknown, clinical suspicion Relapsing polychondritis
Patient 22†	–	4.64	–	5.90 (CMV)	–	1	Panuveitis	–	+	+	+	–	Unknown

EBV, Epstein–Barr virus; PCR, polymerase chain reaction; GWC, Goldman–Wittmer coefficient; KP, keratic precipitates; AU, anterior uveitis; syn, synechia; IS, immunosuppression; HIV, human immunodeficiency virus; POL, punched out lesions; LTBI, latent tuberculosis induced uveitis; HLA-B27, human leukocyte antigen-B27; 0, not performed; CMV, cytomegalovirus; RV, Rubellavirus; HSV, Herpes simplex virus; VZV, Varicella zoster virus; IgG, immunoglobulin G.

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

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

suggesting concurrent active systemic EBV infection.[1,3,4,19]

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

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

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

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

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

SUPPLEMENTARY MATERIAL

Supplemental data for this article can be accessed on the publisher's website.

CONFLICT OF INTEREST

This study was financially supported by AbbVie, the Netherlands. The sponsor had no role in study design, in the collection, analysis and interpretation of data or in the writing of the report, and in the decision to submit the article for publication.

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