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ORIGINAL ARTICLE

Molecular and Serological Intraocular Fluid Analysis of *Coxiella burnetii*-seropositive Patients with Concurrent Idiopathic Uveitis

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ABSTRACT

Purpose: Previous studies have suggested a link between Q fever and uveitis. We determined whether *Coxiella burnetii* causes intraocular infection in *C. burnetii*-seropositive patients with idiopathic uveitis.

Methods: From a retrospective observational case series, paired aqueous humor and serum samples from 10 *C. burnetii*-seropositive patients with idiopathic uveitis were examined for intraocular antibody production by using the Goldmann-Witmer coefficient and by polymerase chain reaction (PCR).

Results: Although intraocular IgG against *C. burnetii* was detected, no intraocular antibody production was observed (low Goldmann Wittmer coefficients). All PCR results were negative.

Conclusions: Uveitis due to an intraocular infection with *C. burnetii* is unlikely.

Keywords: Aqueous humor analysis, *Coxiella burnetii*, infection, uveitis

Uveitis may be caused by intraocular infection or systemic (autoimmune) disease,[1,2] but remains idiopathic in approximately a quarter of cases. Intraocular fluid analysis by both polymerase chain reaction (PCR) and detection of intraocular antibody production is of great value to distinguish infectious from noninfectious uveitis.[3] Moreover, it has led to the discovery of new and putative infectious causes of uveitis.[4,5]

Several cases have been described suggesting that *Coxiella burnetii*, the causative agent of Q fever, may be involved in the development of uveitis.[6,7,8,9,10] In addition, one study reported the identification of 9 *C. burnetii* seropositive cases in a large cohort of 1520 idiopathic uveitis patients. While *C. burnetii*-specific PCR analysis of anterior chamber fluid of patients with a positive Q fever serology was negative, the authors hypothesized that *C. burnetii* caused uveitis in these cases in an antibody-mediated manner.[11,12] Determination of intraocular antibody production against *C. burnetii*, which could confirm this hypothesis, has not been reported yet. From mid 2007 till 2010 a Q fever epidemic occurred in the Netherlands which was, with over 4000 reported acute Q fever cases (which is an underestimation of the real magnitude of the outbreak), unmatched worldwide in size and duration.[13] This event allowed us to investigate the association between *Coxiella burnetii* and uveitis more closely. Using PCR and Goldmann-Witmer coefficient (GWC) analysis to detect intraocular antibody production, we set out to provide evidence for or against the hypothesis that *Coxiella burnetii* is causally related to uveitis.

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METHODS

Patients and Samples

Patients were selected from a database of paired aqueous humor and serum samples that were collected for diagnostic purposes between 1 January 2008 and 31 December 2010 at the University Medical Center Utrecht, a nationwide reference center for the diagnosis of infectious uveitis. The intraocular fluid samples had been stored at −80 °C within 5 h of collection prior to processing for laboratory analysis. All samples had been analyzed by PCR and GWC analysis for at least herpes simplex virus 1 and 2, varicella zoster virus, and *Treponema pallidum*, and in case of posterior uveitis also *Toxoplasma gondii*. We hypothesized that putative cases of *C. burnetii*-induced uveitis would be far more likely to occur in those patients where no other infectious cause for the uveitis has been found. Patients under the age of 18 years or with a laboratory-confirmed infectious uveitis caused by cytomegalovirus, herpes simplex virus types 1 and 2, varicella zoster virus, rubella virus, or *Toxoplasma gondii* were excluded. HLA-B27-positive patients and patients with sarcoidosis were not excluded, as in these cases an infectious trigger has been suggested.

To identify possible cases of uveitis and simultaneous *C. burnetii* infection, each patient's municipality of residence and year of first presentation with uveitis was compared to epidemiological data on the incidence of Q fever. Serological screening for Q fever was performed in all those cases where patients presented with uveitis while their municipality of residence was affected by the epidemic. Intraocular fluids from all *C. burnetii*-seropositive patients with idiopathic uveitis were analyzed by PCR and intraocular antibody production analysis. This study was performed according to the tenets of the Declaration of Helsinki and in agreement with the regulations of the institutional review board of the Utrecht University Medical Center (UMCU). All patients consented to the use of their materials for research purposes.

Antibody Detection

Patients were screened for *C. burnetii*-specific IgG in serum using the Panbio *Coxiella burnetii* (Q fever) IgG ELISA (Panbio, Brisbane, Australia) according to the instructions of the manufacturer. Intraocular antibody production was determined by using the GWC analysis essentially as described previously.[3] *C. burnetii* IgG titers were determined by analyzing 4 serial fourfold dilutions starting at 1:101 for serum and 4 serial twofold dilutions starting at 1:50.5 for intraocular fluid using the Panbio *Coxiella burnetii* (Q fever) IgG ELISA. IgG titers were determined by calculating the dilution at which the extrapolated linear part of the ELISA curves crossed the cutoff value. Total IgG concentrations were determined by an in-house assay as described previously.[3] The GWC was calculated as follows ([specific IgG eye/total IgG eye]:[specific IgG serum/total IgG serum]). Values exceeding 3 are considered indicative of intraocular antibody production.[3]

PCR Analysis

Taqman PCR analysis was performed as described previously.[3] Bacterial DNA was extracted from 12.5 µL of ocular fluid using the MagNa Pure LC Total Nucleic Acid isolation kit – large volume (Roche, Almere, The Netherlands). To monitor the quality of the extraction and the subsequent amplification procedure a standard dose of phocine herpesvirus type 1 (PhHV-1) was added to each sample as an internal control prior to extraction.[14] The DNA was collected in a volume of 100 µL, and per PCR reaction 10 µL was added. The primers and probe for PhHV-1 DNA amplification were described previously.[3] *C. burnetii* DNA was amplified using forward primer (5′-AAAACGGATAAAAAGAGTCTGTGGTT-3′), reverse primer (5′-CCACACAAGCGCGATTCAT-3′) and probe (5′-FAM-AAAGCACTCATTGAGCGCCGCG-TAMRA-3′) as described in[15] Taqman PCR was performed on an ABI Prism 7900 HT sequence detection system (Applied Biosystems, Foster City, CA, USA). The analytical sensitivity of the assay was determined to lie between 10 and 30 copies per reaction. The analytical specificity is 100%. All samples were analyzed in duplicate. None of the samples were inhibited (Tilburg 2010).

RESULTS

A total of 773 adult patients suffering from presumed noninfectious uveitis were included, 245 of which had an elevated risk of *Coxiella* infection, as was determined by linking municipality to the epidemiological data of the Dutch Q fever epidemic. Of these 245 patients, 85 had a uveitis of undefined etiology, 3 of which had been found *C. burnetii* seropositive previously. Of the remaining 82 patients, sufficient serum for *C. burnetii* antibody screening was available in 72 cases. Of these, 8 were seropositive, yielding 11 *C. burnetii*-seropositive patients with idiopathic uveitis.

In 10 cases sufficient ocular fluid was available for GWC determination. Intraocular IgG and thus the GWC were negative in 8 patients. In 2 cases (1 and 9), intraocular *C. burnetii*-specific IgG was positive, but the GWC did not exceed 3, indicating that

TABLE 1. Clinical characteristics and laboratory results of *Coxiella burnetii*-seropositive patients with idiopathic uveitis.

	Gender	Age	Year of sampling	Clinical diagnosis	Laterality	Ocular IgG titer	GWC	PCR
1	Female	23	2010	Acute multifocal placoid pigment epitheliopathy	Unilateral	1:387	1.96	Neg
2	Male	80	2010	Idiopathic panuveitis and fever	Bilateral	Neg	Neg	Neg
3	Female	39	2009	Idiopathic anterior uveitis	Bilateral	Neg	Neg	Neg
4	Female	69	2009	Anterior uveitis and sarcoidosis	Bilateral	Neg	Neg	Neg
5	Female	51	2009	Nodular scleritis and aortitis	Unilateral	Neg	Neg	nd
6	Male	43	2009	Idiopathic chorioretinitis	Unilateral	Neg	Neg	Neg
7	Male	60	2009	Idiopathic panuveitis	Bilateral	Neg	Neg	Neg
8	Male	49	2009	Idiopathic panuveitis	Unilateral	Neg	Neg	Neg
9	Female	48	2010	Panuveitis and papillitis	–	1:51	2.86	nd
10	Male	66	2009	Idiopathic neuroretinitis	Unilateral	Neg	Neg	Neg

GWC, Goldmann-Witmer coefficient; PCR, polymerase chain reaction; nd, not determined.

TABLE 2. Clinical characteristics of patients with concurrent uveitis and *Coxiella burnetii* seropositivity reported in literature.

Article	Gender	Age	Country	Q fever	Uveitis characteristics	Uveitis entities excluded
[6]	Female	33	France	No	Anterior unilateral	Toxoplasmosis, rheumatism, TB
[7]	Male	22	France	Yes	Posterior bilateral	Unspecified
	Female	75	France	No	Posterior unilateral	Unspecified
[10]	Male	44	Spain	Yes	Choroidal neovascularization	Unspecified
[9]	Male	58	France	Yes	Anterior unilateral	Syphilis, TB, sarcoidosis
Million et al. 2010	Male	56	France	No	Posterior bilateral, optic neuritis	Syphilis, *Bartonella henselae/quintana*, *Borrelia*
[11,12]	Male	43	France	Unknown	Posterior unilateral	Unspecified
	Female	39	France	Unknown	Anterior unilateral	Unspecified
	Female	69	France	Unknown	Anterior unilateral	Unspecified
	Female	57	France	Unknown	Anterior bilateral	Unspecified
	Male	76	France	Unknown	Anterior bilateral	Unspecified
	Female	86	France	Unknown	Anterior unilateral	Unspecified
	Male	88	France	Unknown	Posterior unilateral	Unspecified
	Female	76	France	Unknown	Posterior unilateral	Unspecified
	Male	59	France	Unknown	Anterior unilateral	Unspecified

Coxiella-specific IgG was not produced locally, but had leaked from the peripheral blood into the aqueous chamber. PCR analysis was possible on the remainders of 7 aqueous humor samples, but positive results were not found. The clinical characteristics and laboratory results of the examined *C. burnetii*-seropositive patients are listed in Table 1.

DISCUSSION

The present study is the first to examine intraocular *C. burnetii* infection by analysis of intraocular fluid samples using both GWC and PCR. Neither intraocular antibody production nor *C. burnetii* DNA was detected in seropositive patients identified from a cohort of patients with idiopathic uveitis living in the 2007–2010 Q fever epidemic regions in the Netherlands, suggesting that *C. burnetii* was not the causative agent of the uveitis. Obviously, this study is hampered by the relative low number of *C. burnetii*-seropositive uveitis patients analyzed.

Assuming *C. burnetii* uveitis does exist, these results could be explained by a low intraocular bacterial load, concomitant with undetectable intraocular antibody production. False-negative antibody results could arise if only anti-phase I antibodies are produced intraocularly, whereas the ELISA applied here detects anti-phase II antibodies. However, this is unlikely, as anti-phase II antibodies are present during both the acute and chronic phases of the disease, whereas anti-phase I antibodies are predominant in chronic disease.[16] Alternatively, *C. burnetii* uveitis could result from an autoimmune or autoinflammatory response triggered by the bacterial infection, which is not identifiable by GWC or PCR.

On the other hand, Q fever-associated uveitis may not exist at all. Six *Coxiella*-seropositive patients with uveitis have been described in 5 medical case reports,[6,7,8,9,10] and an additional 9 cases were recognized in a large survey.[11,12] These 15 cases, summarized in Table 2, show varying types of uveitis, occurring either during episodes of Q fever or in asymptomatic seropositive patients, as was the case in our patient series. All but one of the previously

described cases concern patients from France, a country where Q fever is endemic to many rural regions. Also, in the described cases, it is often unclear if other possible causes of uveitis were ruled out. The combined data, therefore, support the hypothesis that coexistence of Q fever and uveitis is merely a chance finding.

In conclusion, although it cannot be ruled out that *C. burnetii* is associated with uveitis, the current combined data suggest that intraocular infection with *C. burnetii* is an unlikely cause of uveitis.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Author contributions: Involved in study design (LEH, JJO, LMK, JDFG); conduction of study and data collection (LEH, LMK, JD, JO); analysis and interpretation of data (LEH, JD, JDFG); drafting manuscript (LEH); review and approval of manuscript (JJO, LMK, JO, AR, JDFG).

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