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Preliminary studies of prevalence of *Borrelia burgdorferi* sensu lato and *Toxoplasma gondii* infections markers in patients with encephalitis of unknown aetiology

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Abstract

Introduction and Objective. The aetiology of encephalitis frequently remains undetermined. Infections of the central nervous system (CNS) with *Borrelia burgdorferi* sensu lato and *Toxoplasma gondii* are particularly challenging to confirm due to inconsistent serological findings and low sensitivity of polymerase chain reaction (PCR) in cerebrospinal fluid (CSF). The chemokine CXCL13 has been proposed as a supportive marker for neuroborreliosis. The aim of the study is to investigate the presence of immunological and molecular markers of *B. burgdorferi* s.l. and *T. gondii* in patients with encephalitis of unknown origin, and to evaluate their potential association with CNS involvement.

Materials and Method. A retrospective analysis was conducted on serum and CSF samples from 31 patients with a clinical diagnosis of encephalitis of unknown aetiology. Anti-*Borrelia* and anti-*Toxoplasma* antibodies and CXCL13 levels were measured using ELISA. Pathogen DNA was assessed by real-time PCR.

Results. Anti-*Borrelia* antibodies were detected in 12 (38.7%) serum samples. Elevated CXCL13 levels (>100 pg/mL) were observed in 2 CSF samples. Anti-*T. gondii* IgG was found in 14 (45.2%) serum samples, with 5 (16.1%) also positive in CSF. High-avidity IgG was present in 9 (29.0%) serum and 3 (9.7%) CSF samples. Low-avidity IgG was found in 3 cases, with variable IgM status. No *Borrelia* or *T. gondii* DNA was detected.

Conclusions. The presence of antibodies against *B. burgdorferi* s.l. and *T. gondii* in serum and/or CSF, occasionally accompanied by elevated CSF CXCL13, suggests a potential role of these pathogens in encephalitis of unknown aetiology, even in the absence of detectable DNA, and highlights the diagnostic value of combining serological and molecular testing.

Key words

DNA, *Toxoplasma gondii,* encephalitis, cerebrospinal fluid, *Borrelia burgdorferi* s.l, serological markers

INTRODUCTION

Encephalitis is the result of a focal or diffused inflammatory process that involves the brain parenchyma and has many distinct aetiologies. The clinical manifestations of encephalitis can be mimicked by acute stroke, metabolic or inflammatory disorders, and neoplasia [1]. Although the causes of encephalitis include toxic, autoimmune and post-vaccination reactions [2], the most important factors are viral (e.g., human herpes viruses type 1 – HSV, human adenovirus – HAdV, varicella-zoster virus – VZV, Epstein-Barr virus – EBV, cytomegalovirus – CMV, tick-borne encephalitis virus – TBEV), bacterial (e.g., *Listeria monocytogenes, Mycoplasma spp.*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Borrelia burgdorferi* sensu lato (s.l.) mainly *Borrelia. garinii*), parasitic (*Naegleria fowleri*, *Acanthamoeba* spp) or fungal (*Coccidioides immitis*, *Histoplasma capsulatum*, *Cryptococcus neoformans)* infections [2, 3]. The diagnosis of encephalitis is based on the clinical picture of the disease, the routine peripheral blood cells and CSF laboratory tests, and the presence of serological and molecular markers of neurotropic pathogens [3]. Importantly, for 60% of diagnosed encephalitis cases worldwide, the etiological agent was not identified [2, 3].

Borrelia burgdorferi s.l. and *Toxoplasma gondii*, the aetiological agents of Lyme borreliosis and toxoplasmosis, respectively, are among the pathogens that can cause encephalitis; however, anti-*T. gondii* is routinely tested only in immuno-compromised patients. Both pathogens are spread worldwide and represent a major epidemiological problem, especially in rural and forest areas [4]; nevertheless, climate and environmental changes also facilitate their spread in urban areas [4, 5, 6]. The main reservoirs of *B. burgdorferi* s.l. are small rodents (mice, voles) and deer, while for *T. gondii* – many mammals, including small rodents and birds [5, 6].

The encephalitis caused by *B. garinii* (which demonstrates high tropism to the nervous system cells) is more frequently

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described than that caused by *Borrelia afzelii,* or *B. burgdorferi* sensu stricto (s.s.) [7].

Lyme Neuroborreliosis (LNB) progressing as encephalitis is uncommon and affects subjects in the late stage of infection. Unawareness of spirochetae infection may have diagnostic and therapeutic implications in encephalitis patients, thereby compromising treatment outcomes [8]. Meningoencephalitis is also a common complication in the course of *Borrelia miyamotoi* disease that have been reported in the USA, Japan, and Europe [9]. There are no studies in the literature addressing the prevalence of anti-*B. burgdorferi* s.l. antibodies in patients with encephalitis.

Central nervous system (CNS) infections with *T. gondii* are usually asymptomatic. Clinically overt life-threatening reactivation of the infection usually occurs in immunocompromised patients and is a consequence of rupture of *Toxoplasma* cysts in the brain [7]. In HIV-infected patients, 10–40% are reported to be infected with *T. gondii* (with serological and/or molecular markers of infection), of whom approximately 30% may develop encephalitis [10].

Chemotactic activity of the chemokine CXCL 13 can be used to assess the inflammatory process in the brain in some infections, including *B. burgdorferi* s.l. or *T. gondii* [11, 12, 13]. Previous studies indicate that Lyme disease or toxoplasmosis patients may experience an increase in its concentration in the cerebrospinal fluid, while its serum level remains unchanged [11, 13].

Although considerable progress has been made in understanding the immunopathogenesis of infection, the mechanisms of the parasite's neuroinvasion remains poorly understood [12]. Therefore, the aim of the is to identify *Borrelia* and/or *T. gondii* infection as possible causative agents of encephalitis in patients diagnosed with encephalitis of unknown aetiology [11, 13].

The aim of the study, therefore, was to investigate the presence of immunological and molecular markers of *B. burgdorferi* s.l. and *T. gondii* in patients with encephalitis of unknown aetiology [11, 13], and to evaluate their potential association with central nervous system involvement.

MATERIALS AND METHOD

Study population. A retrospective study was conducted on adult patients hospitalized in the Department of Infectious Diseases for Adults at the Medical University of Warsaw, with diagnosed encephalitis: 18 (52%) women and 13 (48%) men, average age 40.1 ± 15.5 years. Anonymized blood serum and cerebrospinal fluid (CSF) samples were collected during 2013–2014.

The clinical manifestations of encephalitis were: fever (41.9%), headache (38.7%), muscle pain (35.5%), epileptic episodes (22.6%), body numbness (19.4%), behavioural (16.1%), consciousness (16.1%) and speech (9.7%) disorders, as well as joint pain (9.7%), classified as characteristic of encephalitis (information derived from clinical data).

The routine (serological and molecular) testing for possible etiological agents: herpes simplex virus (HSV), tick borne encephalitis virus (TBEV), Epstein-Barr virus (EBV), human herpes virus 1 (HHV1), human herpes virus 2 (HHV2), human herpes virus 6 (HHV6), varicella zoster virus (VZV), cytomegalovirus (CMV), human adenovirus (HAdV), revealed negative results. Diagnostic assessments were performed immediately after sampling in 2013–2014 (information derived from clinical data).

Ethics approval and consent to participate. The study protocol followed the ethical guidelines of the 2013 Declaration of Helsinki, and approved by the Internal Review Board of the Warsaw Medical University (No. KB/97/2013). All ethical approvals were obtained according to Polish regulations.

Written informed consent was obtained from all patients or from close relatives if the

patient was unable to give consent due to her/his condition. However, this consent had to be confirmed once this condition improved.

Laboratory measurements. The laboratory analyses of the markers described in this study were performed during 2022–2023. The presence of anti-Borrelia (IgM and IgG) antibodies in serum and CSF was tested by the Mikrogen Borrelia ELISA recombinant antigen test (Mikrogen Diagnostik GmbH, Neuried, Germany). All positive and equivocal results were verified by the Western blot confirmatory test (RecomLine Borrelia IgM/IgG, Mikrogen Diagnostik GmbH, Neuried, Germany) according to the recommendations of The Polish Society of Epidemiology and Infectious Diseases.

IgM and IgG *T. gondii* antibodies were determined in all serum and cerebrospinal fluid samples. IgG avidity was determined only for IgG-positive samples. The presence of *T. gondii* antibodies was determined using NovaLisa *T. gondii* IgM μcapture and NovaLisa *T. gondii* IgG, as well as the NovaLisa Avidity *T. gondii* IgG tests (NovaTec Immunodiagnostica GmbH, Frankfurt, Germany), according to the manufacturer's instructions and interpretation criteria.

The level of the chemokine CXCL13 in CSF was determined by ELISA (Euroimmun AG, Lübeck, Germany).

The DNA of the pathogens were detected using the real time PCR method. Genomic DNA was isolated from CSF and serum using the DNeasy Blood and Tissue kit (Qiagen, Crawley, UK). Detection of *Borrelia* DNA was performed by GeneProof Borrelia PCR Kit (Brno, Czech Republic), which can identify 15 *B. burgdorferi* s.l. species as well as *B. miyamotoi* (sensitivity of 98.5%). For *Toxoplasma*, DNA identification in CSF and serum, AmpliSens *Toxoplasma gondii*-FRT PCR kit for IVD (E. coli Dx, s.r.o. Prague, Czech Republic) was used according to the manufacturer's instructions and interpretation criteria. Analysis of results was performed by the software of the real-time PCR instrument by measuring fluorescence signal from 2 channels: (1) the signal of the IC DNA amplification product was detected in the channel for the FAM fluorophore; (2) the signal of the *T. gondii* DNA amplification product was detected in the channel for the JOE fluorophore. Results were interpreted by analysing the fluorescence curves. Samples were considered positive if Ct (cycle threshold) values did not exceed 40 cycles.

RESULTS

Overall, anti-*B. burgdorferi* s.l. IgM and/or IgG were found in 12 (38.7%) serum samples. Exclusively IgM and IgG antibodies have been found in 7 (22.6%) and 4 (12.9%) serum samples, respectively. In one case (3.2%), both IgM and IgG antibodies were identified. No antibodies were detected

in the CSF (Tab. 1). No genetic material of *B. burgdorferi* s.l./*B. miyamotoi* was found in any of the samples tested.

Elevated levels of the chemokine CXCL13 in CSF, indicating suspected neuroborreliosis (i.e., >100 pg/ml), were found in 2 samples (6.4%) and increased levels (i.e., 30–100 pg/ml) in 2 cases (6.4%). In the remaining cases, the results were borderline (one case, 20–30 pg/ml) or below the value indicative of neuroborreliosis (<20 pg/ml, 26 cases). There was no significant difference in CXCL13 levels between the patients with presence/absence of serum antibodies to *B. burgdorferi* s.l. (Tab. 1).

In the case of anti-*T. gondii,* IgM antibodies were found in only one serum and CSF sample (3.2%) (Tab. 1). IgG were found in 14 (45.2%) serum samples, of which in 5 (16.1%) cases also in CSF.

The high-avidity IgG (i.e., more than 60%) was observed in 9 (29.0%) serum and 3 (9.7) CSF cases. The low avidity IgG (i.e., lower than 11%) was revealed in 3 cases (one in serum in the presence of IgM, one in serum and CSF in the absence of IgM, and one in the CSF in the absence of IgM). In the remaining cases, the avidity was found to be equivocal (i.e., in the range of 45–60%).

No *Toxoplasma gondii* DNA were detected in any of the analysed patients.

The concurrent presence of anti-*B. burgdorferi* s.l. as well as anti-*T. gondii* antibodies was found in 5 patients (Nos. 2, 8, 20, 23 and 25) (Tab. 1). Patient No. 2 showed the anti-*B. burgdorferi* s.l. IgM (in serum) and high-avidity *T. gondii* IgG (in serum and CSF). Patient No. 20 showed the simultaneous presence of serum anti-*B. burgdorferi* s.l. IgM and IgG and high-avidity anti-*T. gondii* IgG in serum and CSF. In both of these cases, high levels of the chemokine CXCL13 were also demonstrated (Tab. 1).

In the remaining cases, serum anti-*B. burgdorferi* s.l. IgM was accompanied by high avidity anti-*T. gondii* IgG in serum and CXCL13 levels <20 pg/ml.

Table. 1. Prevalence of serological markers of *Borrelia burgdorferi* s.l. and *Toxoplasma gondii* infection in serum and cerebrospinal fluid (CSF) samples and CXCL13 in CSF from patients with encephalitis of unknown etiology

No of patient	anti-*Borrelia burgdorferi* s.l. antibody				Interleukin CXCL13 level (pg/ml)	anti-*Toxoplasma gondii* antibody					
	serum		CSF			serum			CSF		
	IgM	IgG	IgM	IgG		IgM	IgG	IgG avidity	IgM	IgG	IgG avidity
1	-	-	-	-	<20	pos	pos.	L	pos.	-	-
2	pos.	-	-	-	**>100**	-	pos.	H	-	pos.	H
3	pos.	-	-	-	30–100	-	-	-	-	-	-
4	-	-	-	-	<20	-	-	-	-	-	-
5	-	-	-	-	<20	-	-	-	-	-	-
6	-	-	-	-	<20	-	pos.	E	-	-	-
7	pos.	-	-	-	<20	-	-	-	-	-	-
8	pos.	-	-	-	<20	-	pos.	H	-	-	-
9	-	-	-	-	<20	-	pos.	H	-	pos.	H
10	pos.	-	-	-	30–100	-	-	-	-	-	-
11	-	-	-	-	<20	-	pos.	L	-	pos.	L
12	-	pos.	-	-	<20	-	-	-	-	-	-
13	-	-	-	-	<20	-	pos.	H	-	-	-
14	-	-	-	-	<20	-	pos.	E	-	-	-
15	-	pos.	-	-	<20	-	-	-	-	-	-
16	-	pos.	-	-	20–30	-	-	-	-	-	-
17	-	-	-	-	<20	-	pos.	E	-	-	-
18	-	pos.	-	-	<20	-	-	-	-	-	-
19	-	-	-	-	<20	-	pos.	H	-	-	-
20	pos.	pos.	-	-	**>100**	-	pos.	H	-	pos.	H
21	-	-	-	-	<20	-	-	-	-	-	-
22	-	-	-	-	<20	-	-	-	-	-	-
23	pos	-	-	-	<20	-	pos.	H	-	-	-
24	-	-	-	-	<20	-	pos.	H	-	-	-
25	pos.	-	-	-	<20	-	pos.	H	-	pos.	L
26	-	-	-	-	<20	-	-	-	-	-	-
27	-	-	-	-	<20	-	-	-	-	-	-
28	-	-	-	-	<20	-	-	-	-	-	-
29	-	-	-	-	<20	-	-	-	-	-	-
30	-	-	-	-	<20	-	-	-	-	-	-
31	-	-	-	-	<20	-	-	-	-	-	-

(1) Presence of antibody: positive result (pos.); negative result (-); (2) Interpretation of the ELISA chemokine CXCL13 level (pg/ml): >100 – suspected neuroborreliosis; 30–100 – increased level; 20–30 – borderline result; <20 – no indication of neuroborreliosis); (3) Avidity of anti-*T. gondii* IgG: H – high (>60%), L – low (<45%), E – equivocal (45%–60%)

DISCUSSION

Borrelia burgdorferi s.l. and *T. gondii* are zoonotic pathogens that are widespread worldwide. Results of previous studies indicate that they may also play a role in the epidemiology of encephalitis [13, 14]. CNS inflammation may occur in the acute as well as in the chronic phase of *B. burgdorferi* s.l. and *T. gondii* infection [1, 15]. The clinical picture of encephalitis caused by both pathogens is often atypical [16]. Thus, in patients of unknown etiology, the diagnostic work-up should be expanded to include also tests for the presence of specific markers (antibodies or genetic material) of these pathogens [5]. Unfortunately, routine diagnostics procedures do not cover this kind of testing, which reduces the chances of early diagnosis and appropriate treatment [16].

Analysis of antibodies against *B. burgdorferi* s.l. present in serum showed that those of IgM class (suggesting active infection) were found more frequently than of IgG (22.6% vs 12.9%, respectively). Concomitantly, no specific anti-*B. burgdorferi* s.l. antibodies were detected in the CSF. According to the recommendations, to diagnose encephalitis as a consequence of the spirochete infection, intrathecal production of antibodies specific to *B. burgdorferi* s.l. is necessary. However, in the early phase of the disease, laboratory results may remain negative due to too low levels of antibodies [17]. It is estimated that an elevated anti-*Borrelia* antibody index – determined as the ratio of specific antibodies to total IgG in CSF/serum [6] – may be within normal range in up to 20% of cases in the early stages of neuroborreliosis [6], and the only manifestation of the inflammatory process in the brain is the presence of leukocytic pleocytosis in the CSF and clinical symptoms characteristic of encephalitis [18,19]. Thus, the alternative valuable option for the diagnosis of *Borrelia*-related encephalitis may be assessment of the chemokine CXCL13 concentration in the CSF [19, 20, 21]. Among patients with early stage of neuroborreliosis, an increased level of this chemokine was observed [19]. The chemokine is secreted into the CSF by monocytes and dendritic cells, interacting with cell membrane lipoprotein via Toll-like receptor 2 (TLR 2). Considering that this interaction takes place even prior to the development of the humoral response, CXCL13 appears to be a valuable marker of early infection [19]. In the current study, 2 patients presented high levels of CXCL13 in the presence of anti-*B. burgdorferi* in serum. These results may provide a rationale for diagnosis and treatment [21].

To confirm *B. burgdorferi* s.l. as a cause of encephalitis, the bacterial DNA was analysed in CSF [22]. Although all results were negative, this does not necessarily exclude infection with *B. burgdorferi s.l.,* and the negative outcome may reflect a transient presence or very low quantity of bacterial genetic material. The definitive diagnosis of the *T. gondii* encephalitis is based on the detection of tachyzoites in the brain tissue [23, 24]. Before the advent of molecular biology techniques, the aetiological diagnosis of toxoplasmosis was made almost exclusively based on serological tests. It is important to note that serological tests may be falsely negative in immunocompromised patients or during the early phase of infection [25, 26]. Detection of anti-*T. gondii* IgG in cerebrospinal fluid is observed in 30–70% of patients with toxoplasmic encephalitis [23]. However, CSF often does not show any significant changes, mainly due to the lack of contact with the subarachnoid space [26].

In the current study, the 45.2% seroprevalence of *T. gondii* is within the range of the healthy population described in Central Europe (30–50%) [1, 10] and in Poland (25–70% of the general population) [25]. In one case, IgM antibodies in serum were detected, which is indicative of the early phase or reactivation of *T. gondii* infection [27, 28]. It is advised to observe the patient's clinical condition and, in case of clinical signs indicative of toxoplasmosis, to repeat the diagnostics [24, 28]. Gras et al. (2004) showed that IgM detected using ISAGA (Indirect Solid-Phase Assay for Germ Agglutination) and IFAT (Indirect Fluorescent Antibody Test) persisted for 2 years in 27% and 9% of females, respectively [29]. Therefore, IgM detection is no longer a marker of recent infection, unless it is found in high titers. Thus, the current means of recent infection verification is determination of IgG avidity, a method which relies on the progressive increase in the affinity of the antibody for its target antigen following infection [29]. IgG antibodies appear around 4–6 weeks after infection and are initially characterized by a low avidity, suggesting infection within the last 2 months, but in 2–5% of cases, IgG antibodies can 'mature' longer [27]. A low serum IgG antibody avidity observed in 2 (6.4%) patients, may suggest an early stage of *T. gondii* infection, which could indicate acute infection. In such a situation, careful observation of the ongoing disease process and, if necessary, further diagnostic work-up for toxoplasmosis is recommended [30]. In Poland, there are no specific recommendations, therefore the treatment protocol depends solely on the physician's decision [13].

Highly avidity IgG antibodies indicate past infection or a chronic toxoplasmosis [27]. These were concomitantly present in CSF and serum in three cases suggesting infection of the central nervous system [13]. The possibility of passive diffusion of antibodies across the damaged blood-brain barrier should also be considered [28, 24]. In this case, it seems necessary to test for the presence of parasitic DNA in the CSF [14, 23].

One patient revealed a low avidity anti-*T. gondii* IgG in CSF and high avidity in serum, suggesting a recent CNS infection which might have caused encephalitis. It was demonstrated that serological testing in cerebrospinal fluid (IgM, IgG, and IgG avidity) may yield results that differ from those obtained in serum. The presence of IgG antibodies in CSF, together with low-avidity findings, could suggested a recent infection, while the corresponding serum profile can suggest a past infection. Such discrepancies are likely attributable to local antibody synthesis within the central nervous system, and they may provide important evidence of active neurotoxoplasmosis [31]. Similarly, to the B. *burgdorferi* s.l., no parasitic DNA was detected in CSF and serum which again could be explained by a transient /low level presence of genetic material in encephalitis.

In the current study, antibodies against *B. burgdorferi* s.l. and *T. gondii* were detected in 5 (16.1%) patients. Interestingly, the simultaneous detection of antibodies to both pathogens in some of these patients, occasionally accompanied by elevated CSF CXCL13 levels, may reflect complex immune responses or co-infections that are not fully captured by molecular methods. This finding underscores the limitations of relying solely on molecular diagnostics to assess the intricate dynamics of immune interactions in such cases. This is important, especially since the occurrence of antibodies against both pathogens is not a rare event, considering that Poland is an endemic area for Lyme borreliosis (seroprevalence is estimated at 14.7–50.7%)- and is characterized by relatively high (51.7%) seroprevalence of *T. gondii* infection [31, 32].

CONCLUSIONS

The detection of serological markers of *B. burgdorferi* s.l. and *T. gondii* in serum and/or cerebrospinal fluid (CSF) of patients with encephalitis of unknown etiology suggests a potential causative role of these pathogens and highlights the importance of including such tests in routine diagnostic workflows. Although no bacterial or parasitic DNA was detected in any of the analysed samples, the presence of specific antibodies indicates previous exposure or past infection, which may still be clinically relevant. In particular, the concurrent presence of antibodies against both pathogens in several patients, occasionally accompanied by elevated CSF CXCL13 levels, may point to complex immunological interactions or co-infections that are not captured by molecular assays alone. These findings underscore that, despite the inherent limitations of serological testing – including the inability to definitively confirm active infection – such analyses provide valuable information for interpreting the patient's immunological status, guiding further diagnostic investigations, and informing clinical decision-making. Therefore, serological testing should be considered a complementary tool alongside molecular diagnostics in the assessment of encephalitis of unknown aetiology.

Data availability. The datasets used and/or analysed during the study are available from the corresponding author upon reasonable request.

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