Advances in Medical Sciences 63 (2018) 167–172

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article

Tick-borne infections and co-infections in patients with non-specific symptoms in Poland
Tick-borne infections and co-infections

Justyna Dunaj[a], Anna Moniuszko-Malinowska[a,*], Izabela Swiecicka[b], Martin Andersson[c], Piotr Czupryna[a], Krzysztof Rutkowski[d], Grzegorz Zambrowski[b], Joanna Zajkowska[a], Sambor Grygorczuk[a], Maciej Kondrusik[a], Renata Świerzbińska[a], Sławomir Pancewicz[a]

[a] Medical University of Białystok, Department of Infectious Diseases and Neuroinfections, Żurawia 14 E, 15-540 Bialystok, Poland
[b] University of Bialystok, Department of Microbiology, Ciołkowskiego 1J, 15-245 Bialystok, Poland
[c] Center for Ecology and Evolution in Microbial Model Systems (EEMiS), Linnaeus University, SE-391 82 Kalmar, Sweden
[d] Department of Allergy, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom

ARTICLE INFO

Article history:
Received 10 March 2017
Accepted 26 September 2017
Available online xxx

Keywords:
Co-infection
Non-specific symptoms
Anaplasma phagocytophilum
Babesia spp.
Borrelia burgdorferi sensu lato

ABSTRACT

Aim: The aim of the study was the evaluation of the frequency of infections and co-infections among patients hospitalized because of non-specific symptoms after a tick bite.

Materials and methods: Whole blood, serum and cerebrospinal fluid samples from 118 patients hospitalised for non-specific symptoms up to 8 weeks after tick bite from 2010 to 2013 were examined for tick-borne infections. ELISA, Western blot and/or molecular biology (PCR; *fla* gene; *16S rRNA*; sequencing) and thin blood smears (MDD) were used. Control group included 50 healthy blood donors. All controls were tested with PCR and serology according to the same procedure as in patients.

Results: Out of 118 patients 85 (72%) experienced headaches, 15 (13%) vertigo, 32 (27%) nausea, 17 (14%) vomiting, 37 (31%) muscle pain, 73 (62%) fever and 26 (22%) meningeal signs. 47.5% were infected with at least one tick-borne pathogen. *Borrelia burgdorferi* sensu lato infection was confirmed with ELISA, Western blot in serum and/or (PCR (*fla* gene) in whole blood in 29.7% cases. In blood of 11.9% patients *Anaplasma phagocytophilum* DNA (*16S rRNA* gene) was detected; in 0.9% patients 1/118 *Babesia* spp. DNA (*18S rRNA* gene) was also detected. Co-infections were observed in 5.1% of patients with non-specific symptoms. *B. burgdorferi* s.l. – *A. phagocytophilum* co-infection (5/118; 4.2%) was most common. In 1/118 (0.8%) *A. phagocytophilum* – *Babesia* spp. co-infection was detected. All controls were negative for examined pathogens.

Conclusions: Non-specific symptoms after tick bite may be caused by uncommon pathogens or co-infection, therefore it should be considered in differential diagnosis after tick bite.

© 2017 Medical University of Bialystok. Published by Elsevier B.V. All rights reserved.

1. Introduction

In tick-endemic areas in Poland people are exposed to various pathogens transmitted by *Ixodes ricinus* ticks: *Borrelia burgdorferi* sensu lato, *Anaplasma phagocytophilum*, *Rickettsia* spp., *Bartonella* spp., *Francisella tularensis*, tick borne encephalitis virus (TBEV), or *Babesia* spp. New tick-borne pathogens, such as *Candidatus* Neoehrlichia mikurensis or *Borrelia miyamotoi* have been reported. In most cases only *B. burgdorferi* s.l. and TBEV infection are considered during the diagnostic process after tick bite. However, other pathogens might be the real cause of symptoms reported by patients. These pathogens are of special importance in immunodeficient patients [1–7].

Lyme borreliosis (LB) is the most commonly diagnosed tick-borne infection worldwide. It is a multi-system disorder, which may affect skin (erythema migrans (EM), acrodermatitis chronica atrophicans, borrelial lymphocytoma), nervous system (neuroborreliosis), joints (Lyme arthritis), heart (Lyme carditis) or eyes (ocular borreliosis). At the beginning of infection diverse nonspecific symptoms usually appear and they are then often the sign of infection. Different species of *B. burgdorferi* s.l.: *B. burgdorferi* sensu stricto, *B. afzelii* and *B. garinii* may lead to a different clinical picture [8–12].

Human granulocytic anaplasmosis (HGA) is caused by *A. phagocytophilum*. Several variants of *A. phagocytophilum* have

* Corresponding author.
E-mail address: annamoniuszko@op.pl (A. Moniuszko-Malinowska).

https://doi.org/10.1016/j.advms.2017.09.004
1896-1126/© 2017 Medical University of Bialystok. Published by Elsevier B.V. All rights reserved.

been described but not all may cause a symptomatic infection in humans. *A. phagocytophilum*, formerly included in the *Ehrlichiaceae* family, now belongs to the *Anaplasmataceae* family (including *Ehrlichia*, *Aegyptianella*, *Neorickettsia*, *Wolbachia*, *Candidatus* Neoehrlichia and *Candidatus Xenohaliotis)*. In 2001, based on the similarity of *16S rDNA* gene sequences (99.1% homology) and *groESL* gene (100% homology), three previously distinct microorganisms: *Ehrlichia phagocytophila, Ehrlichia equi* and human granulocytic ehrlichiosis (HGE) were combined in *A. phagocytophilum* species, which may be pathogenic for humans [13].

In most cases it is an asymptomatic, self-limiting disorder or a mild infection with mild, non-specific flu-like symptoms. Severe HGA occurs occasionally and presents as intravascular coagulation syndrome, thrombosis or kidney failure, atypical pneumonia or gastroenteritis with GI bleeding. Leucopenia, thrombocytopenia, increased activity of aminotransferases are often observed [4,14–16].

Human babesiosis caused by protozoan *Babesia* spp.: *B. microti*, *B. divergens* and *B. venatorum* (all present in Poland) in immunocompetent patients is usually a self-limiting disorder. In most cases only non-specific flu-like symptoms appear. A severe clinical course is observed very rarely. It is linked with high parasitemia, usually in patients with immunological disorders [17,18]. Hepatomegaly, splenomegaly, hemolytic anemia, renal dysfunction with hemoglobinuria, acute respiratory distress syndrome (ARDS), cardiovascular problems or proteinuria may appear [16,19–21].

Various and non-specific symptoms such as headache, fever, sweats, vertigos, nausea, vomiting, muscle pain can appear after a tick bite. They might be a consequence of tick infestation, very early signs of various tick-borne infections, even in cases of suspected meningitis of unknown origin. Prolonged and intense non-specific symptoms may suggest co-infection with tick-borne pathogens, e g. TBEV and *B. burgdorferi* [4,22–26].

Coexistent chronic diseases can influence the severity of a tick-borne infection [25]. Mixed infections can have a more severe clinical course with long lasting sequelae; their diagnosis can be more difficult. *B. burgdorferi* s.l. – *Babesia* spp., *B. burgdorferi* s.l. – *Bartonella* spp., *B. burgdorferi* s.l. – *A. phagocytophilum* co-infections transmitted by ticks result in various intense symptoms and lead to a prolonged and severe LB [5,22–23,27,28].

The aim of the study was to evaluate the frequency of infections and co-infections in patients hospitalized for non-specific symptoms after a tick bite when TBE, EM or neuroborreliosis were not diagnosed. We compared the frequency of symptoms and various laboratory parameters between patients infected with tick-borne pathogens and those with no detectable infection.

2. Material and methods

2.1. Material

118 patients (53 women and 65 men) in mean age 42.11 +/−16.73 years old out of 549 hospitalized in The Department of Infectious Diseases and Neuroinfections of the Medical University of Bialystok from June 2010 to October 2013 with non-specific symptoms after a tick bite were included in the study. In all patients, tick-borne encephalitis (TBE), neuroborreliosis or EM were excluded based on serological serum and cerebrospinal fluid tests (TBE) and absence of intrathecal production of anti-*B. burgdorferi* antibodies (neuroborreliosis). Erythema migrans was excluded based on clinical criteria i.e. lack of typical skin lesion. Clinical information was obtained from patients' medical documentation and a written questionnaire designed for this study. Control group included 50 healthy blood donors.

EDTA-blood was used for May-Grünwald-Giemsa (MGG) smears for *A. phagocytophilum* and *Babesia* spp. detection, and for molecular detection by PCR of *B. burgdorferi* s.l., *A. phagocytophilum* and *Babesia* spp. Serum was used for immunoserological diagnostic (ELISA, Western blot) of *B. burgdorferi* s.l. or TBEV infection. Cerebrospinal fluid was collected from patients with suspected neuroinfection. It was used for a biochemical analysis, detection of anti TBEV antibodies by ELISA and for *B. burgdorferi* s.l. DNA detection.

In all patients laboratory parameters such as: C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), red blood cells count (RBC), hemoglobin (Hb), white blood cell count (WBC), platelet count (PLT), alanine and asparagine aminotransferase activity (ALAT, ASPAT), lactate dehydrogenase (LDH), creatinine, glucose, fibrinogen and bilirubin were measured.

The study was approved by the Bioethical Commission of the Medical University of Bialystok.

2.2. Methods – microscopic blood smear (MGG)

Fresh whole blood was collected in EDTA bottles. Thin blood smears were stained using May-Grünwald-Giemsa method and examined for *A. phagocytophilum* morulae in neutrophils and *Babesia* spp. piroplasms in erythrocytes. Thin blood smear was counted up to 200 white blood cells and at least 500 red blood cells [27,29].

2.3. Methods – DNA extraction

DNA was extracted using the Qiagen DNAeasy Blood and Tissue Mini kit. 200 μl of cerebrospinal fluid was centrifuged for 20 min at 8000 rpm; 200 μl of whole blood was gently mixed before extraction (of each). Purified DNA isolates were frozen at $-20\,^{\circ}$C.

2.4. Methods – A. phagocytophilum *PCR*

To detect *A. phagocytophilum* DNA in vitro, a PCR targeted the *16S rDNA* gene fragment encoding small ribosomal *16S rRNA* subunit was used. Amplification was performed with the *Anaplasma* PCR kit (Blirt-DNA Gdańsk, Poland) in DNA isolates from whole blood. Analysis was conducted according to the manufacturer's instruction (single PCR 2010–2011; nested PCR 2012–2013). Samples collected between 2010 and 2011 were reanalysed using nested PCR. Results confirmed the previous data. Positive results were 227 bp long fragments of the *16S rDNA* gene fragment in single reaction, and in nested PCR the *16S rDNA* gene fragments: 932 bp long in PCR-OUT and 546 bp long in PCR-IN [27]. Positive and negative controls from the kits were used.

At the Laboratory Centre in Kalmar, Sweden *Anaplasma* spp. was detected using primers that amplify a 257 bp long fragment of the *16S rRNA* gene of *Anaplasma* spp., *Ehrlichia* spp. as well as *Neoehrlichia* spp. [30]. The real-time PCR reaction was performed in Light Cycler 480 (Roche, Switzerland), using iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, USA). The primers sequence were: forward 5′-GGGGATGATGTCAARTCAGCAY-3′ and reverse: 5′-CACCAGCTTCGAGTTAAGCCAAT-3′ [30].

Thermal cycling conditions for amplification of *Anaplasma* spp. included an initial denaturation step at 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s. In addition, a melting curve analysis of the amplified products was performed between 60 °C and 95 °C. Samples with a crossing point (Cp) value lower than 40 were considered positive. All positive amplicons were purified with the Wizard® SV Gel and PCR Clean-Up System (Promega, USA) and subjected to Sanger sequencing at a commercial facility (Macrogen Europe, The Netherlands) [30]. All

Download English Version:

https://daneshyari.com/en/article/8368383

Download Persian Version:

https://daneshyari.com/article/8368383

Daneshyari.com