Case Report

First report of Babesia divergens infection in an HIV patient

Luis M. González a,g, Emma Castro b,g, Cheryl A. Lobo c, Alberto Richart d, Raquel Ramiro a, Fernando González-Camacho e, Daniel Luque e, Aurelio C. Velasco f, Estrella Montero a,*

a Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
b Cruz Roja Española, Centro de Transfusión, Madrid, Spain
c Blood Borne Parasites Department, Lindsey Kimball Research Institute New York Blood Center, New York, New York, USA
d Centro de Transfusión de la Comunidad de Madrid, Madrid, Spain
e Unidad de Microscopía Electrónica y Confo, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain
f Departamento de Microbiología, Hospital Universitario San Carlos, Madrid, Spain

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A B S T R A C T

Human babesiosis is a zoonosis primarily transmitted through ixodes ticks and alternatively by routes such as blood transfusions from asymptomatic donors. We report the first case of human babesiosis caused by Babesia divergens in a patient with HIV. This study also focuses on elucidating the possible transmission route of infection in this patient, who received numerous blood transfusions but showed patent symptoms only after splenectomy. A battery of detection tools along with a novel Western-Blot Assay and Enzyme Linked Immunosorbent Assay using the major surface protein of B. divergens (Bd37) as a target were used to evaluate the presence of B. divergens or antibodies against the parasite in samples from the patient and the blood donors involved in this case. A retrospective study of the humoral status against the parasite revealed B. divergens IgG antibodies in one of the implicated donors, but also showed that the patient had been already exposed to the parasite before any transfusion. Thus, this analysis of natural and transfusion transmission routes suggests a pre-existing subclinical babesiosis in the patient.

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Human babesiosis is an expanding zoonosis transmitted through ticks. Babesia microti, B. divergens, B. duncani, B. venatorum, and B. divergens-like organisms (1) are responsible for the human disease, causing a febrile illness similar to malaria. Transmission of B. microti and B. duncani via blood transfusion from infected asymptomatic donors has also been reported. The spread of Babesia through transfusions is increasingly a problem and is one of the most commonly reported transfusion-transmitted infections in the USA. B. divergens is considered the main agent of human babesiosis in Europe (1). Although B. divergens also meets the requirements for potential transmission through transfusion of blood components, no transfusion transmission has been reported yet. We report the first case of babesiosis caused by B. divergens in an immunocompromised patient with HIV.

1. The patient

A 37-year-old man from Spain, newly diagnosed with HIV infection, showed plasma HIV RNA levels of 277,000 copies/mL and a CD4+ T cell count of 178/μL at diagnosis. The patient was admitted to a tertiary University Hospital in Madrid, Spain. Two weeks after starting the antiretroviral therapy, a hemophagocytic syndrome developed in the patient, possibly as part of an immune restoration syndrome, and he received multiple blood transfusions. To control his progressive deterioration, the patient underwent an elective splenectomy. After a slow recovery, the patient showed a new decline in his general condition, without relevant microbiological findings. About three weeks later, the patient was admitted to the ICU with acute respiratory distress syndrome, transfused again and treated empirically with multiple broad-spectrum antibiotics, antifungals and appropriate drugs for Mycobacterium tuberculosis and M. avium-intracellulare, without

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any improvement. On the 3rd day after admission into the ICU, a Giemsa-stained thin film from peripheral blood showed a few intraerythrocytic parasites compatible with Babesia spp. A 156 bp fragment encoded by the variable region V4 of the B. divergens 18S rRNA gene (from thymine 609 to guanine 764) was amplified from the patient’s blood by PCR using F4Bd (5’-tgcggggtatatat-3’) and R1 Mx (5’-ccaaatatgagctac-c3’) primers. The fragment showed 100% identity to B. divergens and differed to B. diergens like (BLASTN 2.2.30) confirming the presence of B. divergens parasites. Specific treatment for Babesia was started immediately with quinine plus clindamycin, later changed to azithromycin and atovaquone for lower toxicity. The patient’s condition showed a clear improvement and he was discharged from the ICU two weeks later. The patient was found free of parasites by PCR in subsequent follow up visits.

Natural (tick) and transfusion-transmission sources of Babesia were considered in an effort to trace the possible route of infection. Cattle and wild mammals are hosts for B. divergens. The patient lived in Spain, and was the owner of a cat but did not have contact with dogs or other domestic and farm animals. The medical history of the patient included frequent travel to various European countries. In particular, a few weeks before his medical crisis, the patient had travelled to Berlin and visited public green parks, but no pasture or wooded areas. Of note, ticks infected with B. microti, B. venatorum and B. divergens have been recently observed in some urban areas from Germany, but the patient did not recall recent tick bites.

Because the patient’s route of infection did not seem to follow the natural tick-mammal route for acquisition of B. divergens, we investigated transfusion-transmitted babesiosis (TTB) as an alternative route. During his hospital stay, the patient received numerous RBC and platelets transfusions. To find a potential source of infection, repository sample sera from 27 blood donors involved in erythrocyte transfusions to the patient were tested retrospectively.

The sequence encoding Asn to Phe of the major surface antigen of B. divergens, Bd37, were cloned into the expression vector pGEX-4T (GE Healthcare Bio-sciences AB, Uppsala, Sweden), expressed in E. coli BL21 (DE) and purified as recombinant Bd37-GST (Glutathione S-Transferase) fusion protein following manufacturer’s instructions. This protein was then used as a target substrate for both Western blot assay and Enzyme Linked Immunosorbent Assay (ELISA) to assess the presence of antibodies in the patient and donors. Indirect Immunofluorescence Assay (IFA) on smears of parasitized RBCs was also performed to detect anti-parasite antibodies. The tests revealed the presence of specific antibodies against B. divergens by IFA and specific anti-Bd37 IgG antibodies by WB and ELISA in just one serum sample of a blood donor from a rural area of Spain (Table 1. Supplementary files S1 and S2). Two days before the Babesia detection by microscopy, the patient had received a packed erythrocytes concentrate from this positive donor, who therefore could be considered as a possible source of infection in our case. Unfortunately, this donor could not be located, preventing us from performing further analysis such as genotyping the infecting strain. A retrospective study was also performed with six pre-transfusion and three post-transfusion sera samples from the patient. Surprisingly, these samples showed the presence of anti-B. divergens IgG by IFA and anti-Bd37 IgG antibodies detected by WB and ELISA in as early as the first blood sample collected at the beginning of his hospital admission. As a consequence, we could not confirm TTB leading to patient’s seroconversion (Supplementary files S1 and S2). Since the patient was Babesia-seropositive before any transfusion, natural acquisition of the infection and the recent visits of the patient to urban green areas were reconsidered. To find out whether the patient had acquired the Babesia infection recently, serologic testing for specific IgM was performed by WB (Supplementary file S2) and ELISA. B. divergens IgM antibodies could not be detected in sera samples from the patient. This could indicate that the B. divergens infection was not recently acquired, but rather was a pre-existing subclinical babesiosis with a low-grade parasitemia that flourished following splenectomy and the immune decline of the patient.

2. Discussion

We have reported what we think is the first case of a patient coinfected with HIV and B. divergens. HIV-derived immunosuppression and the splenectomy probably co-contributed to the clinical flourishing of the babesiosis. The report also focuses on elucidating the transmission route of the Babesia infection to the patient.

The use of the recombinant Bd37 protein in WB and ELISA along with IFA allowed us to identify B. divergens IgG antibodies in one of the implicated blood donors, but also revealed that the patient had been already exposed to the parasite before transfusions. Additional evidence that the infection was not recently acquired was obtained from the lack of B. divergens IgM antibodies in sera samples from the patient.

As for most emerging infectious diseases, many epidemiological parameters remain unknown: the proportion of undetected babesiosis cases, the prevalence of babesiosis in blood donors, and the risk of getting a Babesia infection following blood transfusion is not well established. Babesia infected ticks and their vertebrate hosts are present in urban, rural and natural areas of Europe. Infection by B. divergens and B. venatorum may follow a protracted course, representing a threat to the blood supply which could lead to TTB cases. Significant seroprevalence rates have been documented in Europe although only around 50 cases of Babesia infections have been reported in the continent so far. This low number of cases invites us to think about asymptomatic carriers going undetected or to misdiagnosis of symptomatic patients. Thus, to fully recognize the status of babesiosis in Europe, sensitive and specific assays capable of detecting the infection are needed to provide robust data for diagnosis, track alternative transmission routes of infection, determine seroprevalence rates in the blood donor population, and thus improve the safety of the blood supply.

Table 1

<table>
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<th>Time (mo)</th>
<th>Parasite detection in peripheral blood</th>
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<td>Optical Microscopy</td>
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<td>NC</td>
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(a) No specific studies were done for parasites. (b) In spite of an active search the donor was not found. (c) Pre-transfusion patient’s sera samples and (d) Post-transfusion patient’s sera samples collected during 4 months.
and the positive donor. *B. divergens* smears were performed using intra-erythrocytic forms (A and D) and free parasites (B, C, and E) prepared from *in vitro* human erythrocyte cultures. All sera samples were diluted at 1:128 and the IgG specific antibodies were detected with goat anti-human IgG antibodies conjugated to fluorescein isothiocyanate (FITC). A and B: serum sample of the patient collected at the beginning of hospital admission. C: serum sample of the positive donor. D and E: negative control (NC). Column 1: Infected blood cells or free merozoites captured by differential interference contrast (DIC). Column 2: DAPI-stained nucleic acids (shown in blue). Column 3: Fluorescing intraerythrocytic parasites or isolated free extracellular merozoites probed with IgG antibodies from the sera samples. Column 4 shows all images overlaid.

Supplementary file S2. Serological detection of specific anti-*B. divergens* IgG and IgM antibodies. WB and ELISA were prepared using recombinant Bd37-GST (59.5 kDa), and GST fusion partner for Bd37 (26 kDa). Specificity for the patient’s and donor’s sera against recombinant Bd37 from *B. divergens* was observed by WB. Sera samples were diluted and the specific cutoff was applied at 1:200 for WB by using 150 ng of each of Bd37 and GST recombinant proteins and probing with IgG (A and B) and IgM (C and D). Bd37 (Panel A) and GST (Panel B) recombinant proteins subjected to SDS-PAGE. Lane 1: positive control (PC). Lane 2: the serum sample of the patient collected at the beginning of hospital admission (early January). Lane 3: the serum sample of the positive donor. Line 4: One of the 26 sera samples from negative donors. Lane 5: negative control (NC). Lane 6 a pool of donors infected by *B. microti* (kindly provided by LFKRI New York Blood Center, New York, NY) did not recognize the recombinant Bd37 protein. None of the sera reacted with the recombinant GST protein. E. Detection of *B. divergens* IgG antibodies by ELISA using the positive control (PC), the negative control (NC) and the positive donor sera samples (symbols and lines in black), six pre-transfusion sera samples from the patient collected between early January to mid-February (symbols and lines in grey) and three post-transfusion sera samples collected between late February to early May (symbols and dotted lines in grey). Samples were diluted 1:100 to 1:800.

Although all infected sera showed higher OD values than the NC sample in a dilution-dependent manner at dilutions 1:100, 1:200 and 1:400, no significant differences in reactivity between pre-transfusion and post-transfusion sera were observed.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2015.02.005.

**References**