Hemolytic Anemia Accelerated by *Babesia* spp. Infection in Splenectomized Patient

BRYAN DANILCHUK, SUSAN J LECLAIR

ABSTRACT

A 50-year-old patient presented with severe fatigue, fevers, unexplained weight loss, and night sweats of two -week duration. Within two days, an episode of intravascular hemolysis was noted. Relevant medical history supported a possible diagnosis of Babesia spp. Molecular testing revealed Babesia DNA although the peripheral blood smear did not demonstrate any classic parasite forms. Treatment for Babesia was begun and the patient improved.

ABBREVIATIONS

ALP - alkaline phosphatase, AST - aspartate aminotransferase, CLL - chronic lymphocytic leukemia, HCT – hematocrit, HGB – hemoglobin, LD - lactate dehydrogenase, NRBC - nucleated red blood cell, PCR - polymerase chain reaction, PLT – platelet, PRBCs - packed red blood cells, RBC - red blood cell, RDW - red blood cell distribution width, WBC - white blood cell, Retic, - reticulocyte, Spp. – Species, Tot Bili - total bilirubin, Dir Bili - direct bilirubin

INDEX TERMS

Babesia, hemolysis, hemolytic anemia, splenectomized

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Bryan Danilchuk, BS, MLS (ASCP)^{CM}, *University of Massachusetts, Dartmouth, MA,*

Susan J. Leclair, Ph.D., *Department of Medical Laboratory Science, University of Massachusetts, Dartmouth, MA.*

Address for Correspondence: Bryan Danilchuk, BS, MLS (ASCP)^{CM}, *University of Massachusetts , 285 Old Westport Road, Dartmouth, MA, 02747, 339-927-1224, bdanilchuk@gmail.com*

A 50-year-old male presented with a complaint of increasing fatigue and unexplained weight loss. He had been relatively healthy until the past few weeks when he became increasingly unable to perform the physical aspects of his job and stopped going to work all together. More recently the patient had painful headaches, nightly fevers over 39˚C, and drenching sweats. He did not complain of change in vision, numbness, rash, nausea, vomiting, or abdominal pain. Relevant medical history included past intravenous drug use, a positive HCV antibody test and a splenectomy at the age of 7 years due to the presence of a confirmed but unidentified auto-antibody. Currently, he is an avid hiker and rock climber. He is in constant contact with the family dog that travels with him during his recreational activities and work, and even shares his bed.

The patient was initially given unknown broad-spectrum antimicrobials for possible sinusitis by his primary care physician. When his condition did not improve he was admitted to the hospital where hemolysis was noted. Within two days of admission, the patient's hematocrit decreased significantly and the LD and bilirubin values increased supporting an initial diagnosis of hemolytic anemia. Due to his medical history, the cause of this anemia was first assumed to be an exacerbation of the original auto-antibody. Blood cultures, ordered due to the fever, were negative. In light of his recent social history, initial laboratory tests also included HIV testing which was negative. Due to the degree of outdoor exposure in a Babesia endemic area, he was also tested for Babesia. Because of the potential exposure to ticks, he was started on atovaquone, azithromycin, and doxycycline.

Both thick and thin peripheral blood smear evaluations for *Babesia* spp. were performed and no classic forms were present. Other RBC inclusions such as Howell-Jolly bodies were present and other unidentified material was noted. Figure 1 is a photograph taken from the patient's peripheral blood. The center cell demonstrates the presence of some additional material

that appears to be similar in color to nuclear material but lacks the structure seen in classic presentations of intra-erythrocytic parasites. Due to the variable cross reactivity seen in indirect fluorescent antibody tests, the decision was made to perform a molecular test for direct observation of the presence of *Babesia* spp. In addition, the molecular test has the advantage of demonstrating newer variants of Babesia. A Real-time PCR test for *Babesia* spp. was positive. While this procedure does not distinguish between current and past infections, the patient's current medical presentation supported the interpretation of current Babesiosis.[1]

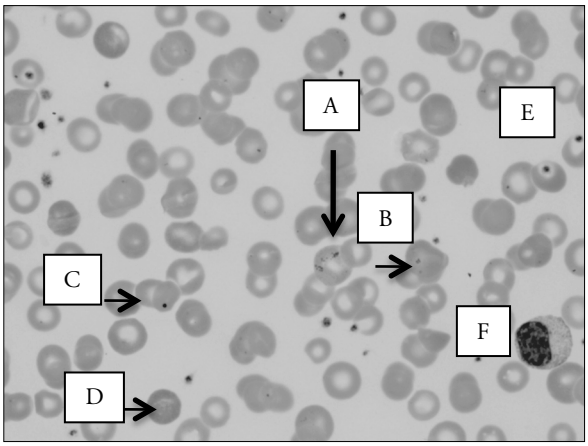

Figure 1. Peripheral blood smear from patient. Note the poorly defined inclusion in the center of the field (A). Notice basophilic stippling (B), Howell-Jolly bodies (C), polychromasia (D), and platelets on RBCs (E). Lymphocyte in lower right corner (F).

After return of the PCR test results, his medication was changed to quinine and clindamycin. The patient improved, had no more fevers, and began to gain back the weight he had lost. He was discharged after eleven days with laboratory values slowly returning to normal. After two weeks, upon completion of medication for Babesiosis, the patient returned for a follow-up appointment to check his hemolytic status and appeared to be in good health.

OVERVIEW

The patient's initial laboratory test work-up indicated a hemolytic episode since his hematocrit dropped from .417 L/L to .270 L/L with no signs of hemorrhage. His LD was consistently around 1500 IU/L, total and direct bilirubin levels at 3.6 mg/dL and 1.2 mg/dL respectively. The hemoglobin was 7.4 g/dL, and he had 80 NRBCs/100WBCs. He had no recorded hemolytic event since his splenectomy at the age of seven. The patient had a positive Direct Antihuman Globulin Test using a poly-specific reagent indicating his RBCs were coated with antibody and/or compliment thus, substantiating the working diagnosis of in-vivo hemolysis.

An elution prepared from the patient's red cells reacted weakly positive (1+) against a panel of RBCs and the patient's own RBCs. This serologic pattern correlates with a low-titer warm auto-antibody, but the extent of the hemolysis seen appeared inappropriate. In addition, a cold agglutinin was detected to react at 6°C and 18°C but not at room or body temperature. Upon further testing it was concluded that this cold agglutinin was non-reactive at 1:16 and had negative thermal amplitude, indicating that it was not clinically significant.[2]

The *Babesia* spp. diagnosis was uncertain until the PCR result returned positive. Another finding of concern was lymphadenopathy in the patient's abdomen and chest, but bone marrow biopsy for potential lymphoproliferative disorders was interpreted as normal. The lymphadenopathy appeared not to be associated with the *Babesia* infection and will be monitored for potential progress.

PATHOGENESIS

Babesiosis is a tick-transmitted disease caused by members of the genus *Babesia*. They are the third most common blood parasite in the world (trypanosomes being first, followed by malaria) and can infect vertebrates via transmission by *Ixodid* spp. ticks.[3] To date, all *Babesia* spp. transmitted have been linked via *Ixodid* spp. ticks and the most common species that infects humans in the United States is *Babesia microti*.[4] The life cycle of this parasite has alternating sexual and asexual reproduction stages. The infective stage (sexual) occurs in the tick where the zygote migrates to the salivary glands. In the salivary glands sporozoites are formed from the zygote.[5] Next the sporozoites are passed from the tick to mammals via a blood meal. Once inside the mammal the sporozoites infect red blood cells, become trophozoites and divide by binary fission (asexual) to become merozoites. These, in turn, are released from the infected RBC and infect other RBCs.[6]

Most *Babesia* spp. infections go undiagnosed because it is primarily self-limiting in most people. It is endemic in the temperate regions of the United States (northeast and Great Lakes region) and in rare cases it can progress to a full-blown, rampant, malaria-like disease, which results in severe hemolysis and occasionally death. It is common that during babesiosis <1% of the RBCs are infected which would make it hard to see the parasite microscopically.[7]

Onset of disease symptoms usually occurs within 1 to 3 weeks of the tick bite and the clinical course tends to be more severe in splenectomized patients. Depending on the degree of infection, laboratory values can include high levels of ALP, unconjugated bilirubin, and LD, as well as thrombocytopenia.[8] The majority of the United States' population who become infected with *Babesia* spp. are between the age of 50-60 years old and had been previously splenectomized.

RELEVANT LABORATORY DATA

It is puzzling that the patient was infected during the winter. *Babesia* spp. infection are not common during the winter months compared to the summer (May-July).[11] The patient began feeling ill and delayed in seeking medical attention until a few weeks after symptoms began. As stated, most *Babesia* spp. infections infect <1% of RBCs, but after multiple examinations and the recognition that the unknown RBC inclusions were unusual presentations of Babesia, the patient was observed to have 6% of his RBCs infected.

By using Real-time PCR, laboratorians were able to measure nucleic acid amplification as it occurs, not at the end of PCR cycles, as in traditional PCR. Real-time PCR can be used for accurate pathogen detection and enables laboratorians to determine the starting concentration of nucleic acid. By use of real-time PCR, clinicians were able to obtain results pertaining to an infection of *Babesia* spp. in the patient.

A rare but major complication of babesiosis is hemolytic anemia. The patient's hematocrit decreased rapidly while iron, LDH, unconjugated bilirubin, and total bilirubin levels significantly increased. The increased reticulocyte count indicated that the patient was producing RBCs rapidly and releasing them into circulation prematurely, another sign of in-vivo hemolysis.

Table 1 (Day 1 represents the day the patient arrived from the outside hospital) indicates that hemolysis is undoubtedly occurring, but during treatment the rate of hemolysis begins to lessen and the patient's laboratory values demonstrate a return to normal. In addition to *in-vivo* hemolysis, the patient's laboratory results correlate with Babesiosis – increased unconjugated bilirubin, ALP, LDH, and thrombocytopenia. Babesiosis in patients who have been splenectomized usually have a weaker defense against the parasite which causes a more serious infection.[12] Milder cases occur in non-splenectomized patients and this may be due to the spleen's ability to remove abnormal RBCs, as well as removing parasites. By not filtering out the infected red cells, the parasite is able continue to reproduce and spread.

Table 1. Patient's laboratory values (Day 1 indicates the day the patient arrived from the outside hospital and Day 5 is results two days after treatment commenced)

Test (reference range)	Day 1 (Arrival)	Day 5	Day 11 (Discharge)	Follow-up appointment
WBC (4.5-11.0 x10^9/L)	15.7	9.1	7.1	10.1
RBC (4.50-5.90 x10^{12}/L)	2.15	2.33	2.80	4.56
HCT (.410-.530 L/L)	.268	.302	-----	.451
HGB (135-175 g/L)	81	101	114	151
PLT (150-400 x10^9/L)	97	142	253	331
RDW (11.5-14.5%)	24.7	-----	-----	-----
Retic (0.5-2.5%)	23.5	18.5	6	1.6
NRBC (0/100 WBC)	80	18	3	-----
Tot Bili (0-17.1 μmol/L)	65.0	22.2	10.3	-----
Dir Bili (0-6.84 μmol/L)	20.5	5.13	3.42	-----
ALP (45-115 IU/L)	114	128	98	-----
AST (10-40 IU/L)	106	119	46	-----
LDH (110-210 IU/L)	1597	1760	828	-----
Serum Iron (45-160 μg/dL)	204	-----	-----	-----
Ferritin (30-300 μg/mL)	8951	-----	-----	-----

Another potential cause of the hemolysis may have had

to do with an enlarged lymph node found in the patient's chest via CT scan. Lymphoma has signs and symptoms of elevated LDH, enlarged lymph nodes, fevers, night sweats, and unexplained weight loss. Hemolytic anemia is seen in chronic lymphocytic leukemia (CLL), but this diagnosis does not correlate with the patient's differential that indicated no immature WBCs. A bone marrow biopsy was performed and results returned normal, enabling physicians to rule out stage IV lymphoma at this time.

TREATMENT AND PROGNOSIS

Babesiosis is often self-limiting, but if treatment is required, patients are given either intravenous or oral clindamycin and oral quinine (an antimalarial drug) which studies had shown to have a positive effect on the disease.[13] Other drugs such as doxycycline and azithromycin may also be prescribed if there is an infection in addition to babesiosis. Another study proved that azithromycin in combination with atovaquone was shown to be successful in a 4-month treatment regime for patients who are asplenic and cannot tolerate quinine. For extreme cases when parasitemia is overwhelming, complete blood transfusions have been performed by apheresis.[15.]

The patient was initially given unknown medication for sinusitis but once a diagnosis of *Babesia* spp. infection was made, he was started on atovaquone, azithromycin, and doxycycline. The causative organism of Lyme disease, *Borrelia burgdorferi,* can also be transmitted by *Ixodid* spp. ticks.[16] Doxycycline is commonly used to treat Lyme disease, so this drug was administered in case the patient was co-infected with *Borrelia burgdorferi*. The patient also received a total of four units of PRBCs during the hemolytic episode. He was continued on atovaquone, azithromycin, doxycycline, quinine, and clindamycin. By the end of his two week stay, the patient had no fevers, night sweats, chills, or abdominal pain. In addition, he had recovered some weight.

His prognosis appeared to be very good since his hemolytic episode was most likely secondary to babesiosis, not his low-titer warm auto-antibody or cold agglutinin. Table 1, Day 11 displays the patient's results on his discharge date. His condition was improved dramatically supporting the theory that the in-vivo hemolysis was not due to lymphoma or an auto-antibody. The patient agreed to return upon completion of medication for another evaluation of his hemolysis status as well as another screen for lymphoma.

CASE CONCLUSION

The patient presented with significant in-vivo hemolysis and possible babesiosis after feeling fatigued, suffering night sweats, and unexplained weight loss.[17] At first, due to his history, it seemed the patient was undergoing an autoimmune hemolytic episode. After laboratory testing, it was demonstrated that he had a low-titer warm auto-antibody as well as a very low cold agglutinin. It was later concluded that these antibody levels were too low to cause hemolysis of this extent.

The patient was diagnosed with babesiosis most probably contracted during one of his hiking treks or from close contact with his dog. He was initially started on medication to treat the babesiosis but as his hemolysis increased he was transfused with PRBCs as needed. Microscopic evaluation of the peripheral blood did not demonstrate *Babesia* spp., however, other red blood cell inclusions appeared to resemble *Babesia* spp. but were not conclusive. The diagnosis was confirmed when the PCR for *Babesia* spp. returned positive. The intense reaction and infection were attributed to his asplenic state. It is possible that if the patient had sought medical attention instead of ignoring his initial symptoms, his hemolytic state could have been avoided. Another cause for concern was enlarged lymph nodes in the patient's chest, but peripheral blood differential and bone marrow biopsy did not correlate with lymphoma.

After two weeks of hospitalization the patient was released and reported to be in good health. His hematocrit was rising and his LDH and NRBCs were decreasing. Upon completion of his medication the patient returned for a follow-up examination and it appeared that his hemolytic anemia had subsided. Thick and thin smears were completed a second time to check for *Babesia* spp. and these returned negative. The patient went back to work and appears to be in good health. He received information from his primary care physician concerning the importance of preventing tick exposure to avoid future life-threatening infections.

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