

Emergence of Resistance to Azithromycin-Atovaquone in Immunocompromised Patients with *Babesia microti* Infection

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Background. Babesiosis is an emerging tickborne malaria-like infection principally caused by *Babesia microti*. This infection typically resolves either spontaneously or after administration of a 7–10-day course of azithromycin plus atovaquone or clindamycin plus quinine. Although certain highly immunocompromised patients may respond suboptimally to these drug regimens, unlike the situation with malaria there has been no reported evidence that the cause of treatment failure is infection with drug-resistant strains of *B. microti*.

Methods. Emergence of drug resistance in *B. microti* was defined as the development of a microbiologic relapse (recurrent parasitemia or a marked increase in parasitemia) in association with both clinical and laboratory abnormalities indicative of active babesiosis in a patient after ≥ 28 days of uninterrupted antibabesia drug therapy and while still receiving treatment.

Results. The clinical case histories of 3 highly immunocompromised patients who received a subcurative course of azithromycin-atovaquone associated with the eventual development of resistance to this drug regimen are described. One of the 3 patients died of complications related to babesiosis.

Conclusions. *B. microti* may become resistant to azithromycin-atovaquone during the treatment of babesiosis with this combined drug regimen in highly immunocompromised patients. Although research is needed to determine the optimal therapy for highly immunocompromised patients with babesiosis, reducing the level of immunosuppression when possible would appear to be a desirable strategy.

Babesiosis is an emerging tickborne malaria-like infection principally caused by *Babesia microti*. This infection typically resolves either spontaneously or after administration of a 7–10-day course of azithromycin plus atovaquone or clindamycin plus quinine [1, 2]. Although certain highly immunocompromised patients may respond suboptimally to these drug regimens, unlike the situation with malaria there has been no reported evidence that the cause of treatment failure is infection with drug-resistant strains of *B. microti* [3]. Furthermore, the emergence of drug resistance during

a course of treatment for babesiosis has not been reported in humans [3]. We describe here 3 highly immunocompromised patients who received a subcurative course of azithromycin-atovaquone associated with the eventual development of resistance to this drug regimen.

METHODS

Emergence of drug resistance in *B. microti* was defined as the development of a microbiologic relapse (recurrent parasitemia or a marked increase in parasitemia) in association with both clinical and laboratory abnormalities indicative of active babesiosis in a patient after ≥ 28 days of uninterrupted antibabesia drug therapy and while still receiving treatment. Three patients known to the authors to have met these criteria are included in this article. Patient 2 was included in a previously published case series [3].

Received 28 May 2009; accepted 31 August 2009; electronically published 4 January 2010.

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Clinical Infectious Diseases 2010;50:381–6

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1058-4838/2010/5003-0012\$15.00
DOI: 10.1086/649859

RESULTS

Patient 1. A 63-year-old man from Westchester County, New York, was electively hospitalized (hospitalization 1; 7 January to 4 February 2008) on 7 January 2008 for splenectomy because of the suspicion of recurrent non-Hodgkin lymphoma. He had been given a diagnosis of stage IV follicular B cell lymphoma with bone marrow involvement in 2003 and was treated with chemotherapy (cyclophosphamide-doxorubicin-vincristine-prednisone [CHOP]) from July to October 2003. He was also treated with rituximab; including maintenance therapy, he received 32 doses beginning on 8 July 2003 and ending on 20 November 2007. A positron emission tomography scan on 9 August 2007 showed increasing splenomegaly since the prior scan on 9 February 2007. The patient experienced a 15-pound weight loss over the 2 months preceding hospitalization, and he had fever (temperatures to 39.4°C) for the prior 2 weeks.

In the hospital, the patient was febrile and had abnormal hematologic parameters before the splenectomy (hemoglobin level, 9.3 g/dL; platelet count, 93,000/ μ L) that persisted postoperatively. Pathologic examination of the spleen did not show lymphoma. A peripheral blood smear on the sixth postoperative day showed *B. microti* (level of parasitemia, 0.5%) (Figures 1 and 2) that prompted review of the blood smear performed before the splenectomy, which also showed *B. microti*. The patient began treatment with quinine (650 mg orally 3 times

a day) and clindamycin (900 mg intravenously 3 times a day) on 13 January 2008. Clindamycin-quinine was discontinued on 15 January 2008 because of a complaint of hearing loss. Azithromycin (600 mg orally once a day) plus atovaquone (750 orally twice a day) were started on 15 January 2008. He defervesced by 19 January 2008. Clinical improvement continued, and peripheral blood smear results were negative for intraerythrocytic parasites on 28, 29, and 30 January 2008. *B. microti* DNA, however, was detected by polymerase chain reaction (PCR) performed by a commercial laboratory on 2 February 2008. He was seronegative for antibody to *Borrelia burgdorferi*.

The patient was compliant with azithromycin-atovaquone therapy, which he received from 15 January to 27 February 2008; to improve tolerability, the dose of azithromycin was reduced from 600 mg once a day to 250 mg once a day on 1 February 2008. He had no clinical signs of having a condition associated with malabsorption. At completion of therapy, the patient felt well and had gained 20 pounds; a blood smear on 21 February 2008 did not show babesia. Fever (temperatures to >38.9°C) recurred, however, on 29 February 2008.

On readmission (hospitalization 2; 7–20 March 2008), the level of parasitemia was 3% (Figure 2). Because of unexplained granulocytopenia (200 neutrophils/ μ L) on that date, the patient received 2 doses of granulocyte colony stimulating factor. The relapse of babesiosis was treated with clindamycin-quinine, but quinine was discontinued 2 days later on 9 March 2008 because

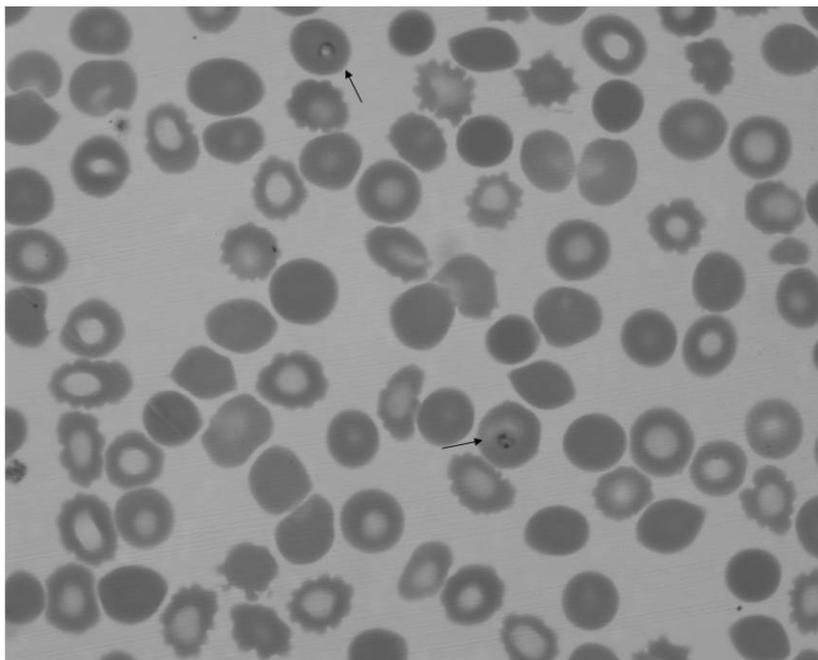


Figure 1. Intraerythrocytic ring forms (arrows) consistent with *Babesia microti* noted on a peripheral blood smear from day 7 of the first hospitalization of patient 1.

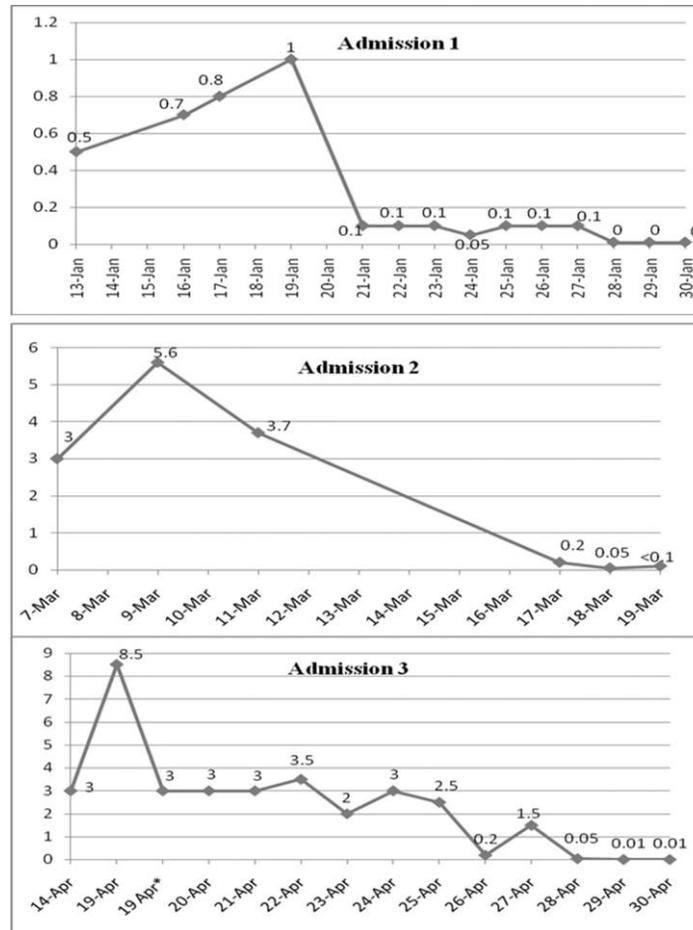


Figure 2. Level of *Babesia microti* parasitemia on selected days of the 3 hospitalizations for patient 1. Red cell exchange transfusions were performed on 12 March and 19 April 2008. The date April 19 is listed twice; the one with an asterisk is for the level of parasitemia after the red cell exchange transfusion performed on that date. The vertical axes show the percentage of erythrocytes infected with *B. microti*.

of intolerance and an increase in the level of parasitemia to 5.6%; atovaquone (750 mg twice a day) was started. Because of persistent fever (temperatures to $>40^{\circ}\text{C}$) and continued severity of illness, he underwent red cell exchange transfusion on 12 March 2008; also on that date, azithromycin (500 mg intravenously once a day) was substituted for clindamycin. Intravenous immunoglobulin (400 mg/kg) was given on 13 March 2008 because of hypogammaglobulinemia (immunoglobulin M [IgM] level, 3 g/L; immunoglobulin A level, 32 g/L; immunoglobulin G [IgG] level, 211 g/L). The patient defervesced by 17 March. The level of parasitemia fell to $<0.1\%$ on 18 March (Figure 2), the same day that the azithromycin dose was changed to 500 mg orally once a day.

After 31 days of azithromycin-atovaquone therapy and while still receiving this treatment, fever with rigors recurred on 11 April 2008 associated with a level of parasitemia of 3% on 14 April 2008 that increased to 8.5% five days later on 19 April 2008, the highest level that had thus far been recorded (Figure

2). These events provided evidence that the strain of *B. microti* infecting this patient was resistant to azithromycin-atovaquone.

The patient was hospitalized again on 18 April (hospitalization 3; 18–30 April 2008). He underwent a red cell exchange transfusion on 19 April 2008. He received a variety of drugs for the babesia infection and was eventually stabilized with a multidrug regimen consisting of malarone (4 tablets daily; each tablet contained 100 mg of proguanil plus 250 mg of atovaquone), atovaquone (750 mg once a day), doxycycline (100 mg orally twice a day), and clindamycin (300 mg orally once a day). He continued receiving the malarone-containing regimen for ~13 months until 21 May 2009, which was 18 months after the last dose of rituximab. This duration of treatment was chosen because a recent study suggested that the effects of rituximab with respect to susceptibility to severe babesiosis might last for this period of time [3]. In addition, the patient has been treated with intravenous immunoglobulin every 2–3 weeks until the present time.

The patient was seronegative for both IgG and IgM to *B. microti* on 2 February 2008 and again on 28 March 2008 by an indirect immunofluorescence assay performed by a commercial laboratory; repeat testing on 21 August 2008 showed an IgM titer of 1:160 but no detectable IgG to *B. microti*.

As of 21 December 2009, 7 months after antibabesiasis therapy was discontinued, our patient felt completely well; his most recent hemoglobin level was 14.1 g/dL, and the platelet count was 514,000/ μ L on 4 December 2009. He has had consistently negative blood smear results since 7 May 2008, although *B. microti* DNA was detectable until 12 August 2008, with the first negative PCR result occurring on 18 September 2008.

Patient 2. An 86-year-old woman from Connecticut presented with fever and anemia on 30 June 2005. The patient had been given a diagnosis of stage IV large cell lymphoma in 1999, at which time she underwent a staging splenectomy. She was treated with CHOP and received 12 doses of rituximab, ending in April 2005.

A diagnosis of babesiosis was made on the basis of a blood smear showing numerous parasites, and the patient was treated with atovaquone (750 mg twice a day) and azithromycin (500 mg on day 1 and then 250 mg once a day for 7 days) starting on 15 July 2005. The patient defervesced and experienced some clinical improvement; a blood smear was not done at the conclusion of the antibabesiasis treatment.

Two weeks after the azithromycin-atovaquone therapy was discontinued, fever (temperature to 38.9°C) recurred associated with chills, sweats, weakness, anorexia, and a 15-pound weight loss, as well as anemia and thrombocytopenia. She was admitted to the hospital on 16 August 2005 and was found to have numerous babesiasis on blood smear. She was treated with clindamycin-quinine, which resulted in a decrease in the level of parasitemia to <1% on 3 September 2005 and resolution of fever. *B. microti* DNA was amplified from blood. Because of the development of a generalized rash, treatment was changed on 3 September 2005 to atovaquone (750 mg twice a day) and azithromycin (500 mg on day 1 followed by 250 mg once a day); in addition, she was given repeated red blood cell transfusions and intravenous immunoglobulin because of persistent pancytopenia.

Evidence that the strain of *B. microti* infecting this patient was resistant to azithromycin-atovaquone appeared on the 49th day of treatment with this drug regimen, when the patient was readmitted to the hospital on 21 October 2005 with recurrent fever and a level of parasitemia of 30%. Two red cell exchange transfusions were performed. Various antibabesiasis therapies were tried sequentially, and she was eventually stabilized with a regimen of clindamycin (450 mg orally 4 times a day), doxycycline (100 mg orally twice a day), atovaquone (750 mg orally once a day), and interferon gamma (84 μ g subcutaneously every other day). Blood smear and PCR results for babesiasis were neg-

ative on 19 June and 12 July 2006. Antibabesiasis treatment was discontinued on 10 July 2006, after completion of >5 months of treatment with the final treatment regimen. Blood smear and PCR results were negative for the next 5 months, and the patient has been asymptomatic up to the time of the last follow-up, 2.5 years after discontinuation of antibabesiasis treatment.

Babesiasis serological analysis in both September and December 2005 showed an IgM titer of <1:16 and an IgG titer of 1:64.

Patient 3. A 45-year-old asymptomatic woman from Connecticut was given a diagnosis of babesiosis (level of parasitemia, 5%) in May 2008. She had a history of Hodgkin disease, which was diagnosed in 1988. She had undergone a splenectomy and had received chemotherapy and radiation therapy. In August 2007, she was found to have IgG2 subclass deficiency. She underwent a combined liver and kidney transplant in October 2007 because of complications of hepatitis C virus infection and was receiving cyclosporine and prednisone.

Because of the babesiosis, the patient was treated with azithromycin (250 mg once a day) plus atovaquone (750 mg twice a day) from 22 May to 31 July 2008. A blood smear result was negative for babesiasis on 11 June 2008, and a PCR result was negative on 28 July 2008. A test result was negative for antibody to *B. microti* on 23 June 2008.

On 15 October 2008, the patient developed fever (temperature, 38.9°C), and *B. microti* was again present on smear (level of parasitemia, <1%). Treatment with azithromycin-atovaquone at the same dosages as before was restarted on 15 October 2008. The fever resolved, although smear results remained positive with a level of parasitemia of <1% through 26 November 2008, except for 1 occasion on 17 November 2008, when a level of 2% was recorded. Evidence for the development of resistance to azithromycin-atovaquone appeared on 4 December 2008 after 51 days of this drug regimen, when the level of parasitemia increased to 7%, which was confirmed on 10 December 2008 (7.7%). Despite changes in antibabesiasis therapy and exchange blood transfusion with resulting decreases in the level of parasitemia, the patient developed multiorgan failure and died on 20 December 2008.

DISCUSSION

The recommended duration of treatment for babesiosis is 7–10 days [1, 2], but this duration of treatment may be inadequate for patients with immunodeficiency [3]. Our experience with 3 highly immunocompromised patients clearly demonstrates the challenges of eradicating babesiosis in such patients. Each of our patients had a history of lymphoma and asplenia; 2 had received rituximab, and 1 had undergone solid organ transplant. In addition, 2 of our patients had hypogammaglobulinemia, and the third had IgG2 subclass deficiency; 1 had transient granulocytopenia. The failure of azithromycin-atovaquone therapy to eradicate babesiosis in these patients serves

to highlight certain limitations of current treatment regimens that are less likely to be appreciated in nonimmunocompromised patients, in whom babesiosis may resolve spontaneously without antiparasitic drug therapy [4, 5].

In each of our patients, babesiosis relapsed after completion of an initial course of azithromycin-atovaquone. Then, when they were re-treated with this regimen for >30 days, the patients experienced relapse while still receiving therapy (Table 1). The patients were compliant with taking the drugs, and none had clinical evidence of malabsorption. Therefore, we conclude that resistance to an azithromycin-atovaquone drug regimen can emerge during treatment. Whether a higher dosage of either or both of the components of this regimen could have overcome the resistance is unknown.

Infection in 2 of the patients appeared to resolve coincidentally with treatment with multidrug regimens that also included atovaquone. Whether the atovaquone provided any clinical benefit in these salvage therapies is unclear, but it is possible that it contributed in an additive or synergistic manner to the treatment benefit afforded by clindamycin or proguanil [6]. In addition, it is conceivable that resistance to azithromycin-atovaquone might be observed clinically even if the actual resistance were only to the azithromycin component.

Emergence of resistance to atovaquone used as a single drug has been documented in animal models of infection using hamsters or gerbils [7, 8]. The mechanism for the development of

resistance was not established. Of potential relevance to our patients, there was no evidence for the emergence of resistance to atovaquone in these animals when given in combination with a second drug.

Observations made in animal models of infection [7–12], which are supported by the events that occurred in the patients described in this article, also suggest that individual drugs (as well as drug combinations) either inconsistently kill the parasite or are exclusively inhibitory against *B. microti*. The observation that low-level parasitemia will often persist after completion of a ≥ 7 -day course of antibabesial treatment in humans with apparently normal immune systems provides additional evidence in support of this hypothesis [2, 5]. Those animal studies in which drug therapy appeared to be curative for *B. microti* infection need to be interpreted cautiously [13]. Misleading conclusions about microbiologic cures can result from terminating experiments before recrudescence would have occurred or from assaying for persistent infection without first subjecting animals to immunosuppression [8, 10]. Thus, it is highly likely that the drug regimens currently used clinically are adjunctive to an effective immune response for the eradication of *B. microti* infection.

The association between persistent babesiosis and therapy with rituximab (a monoclonal antibody that depletes B cells) would seem to imply that humoral immunity is of considerable relevance to an effective host response in babesiosis [3]. Two

Table 1. Evidence for the Development of Resistance to Azithromycin plus Atovaquone in 3 Highly Immunocompromised Patients with Babesiosis

Patient	Initial dosage regimen	Outcome	Subsequent dosage regimen ^a after relapse	Outcome
1	Atovaquone at 750 mg orally twice a day for 44 days plus azithromycin at 600 mg orally once a day for 17 days and then at 250 mg orally once a day for 27 days	Defervesced; 20-pound weight gain; negative blood smear result; experienced relapse with fever 2 days after treatment was discontinued; level of parasitemia of 3%	Atovaquone at 750 mg orally twice a day plus azithromycin at 500 mg intravenously for 5 days, then atovaquone at 750 mg orally twice a day plus azithromycin at 500 mg orally once a day	After 31 days of azithromycin-atovaquone and <i>while receiving this treatment</i> , experienced relapse with fever; level of parasitemia increased from <0.1% to 8.5%
2	Atovaquone at 750 mg orally twice a day for 7 days plus azithromycin at 500 mg orally for 1 day and then at 250 mg orally once a day for 6 days	Defervesced with some clinical improvement; posttreatment smear not done; experienced relapse with fever and weight loss 2 weeks later; parasites detected on smear	Atovaquone at 750 mg orally twice a day plus azithromycin at 500 mg orally for 1 day and then at 250 mg orally once a day	After 49 days of azithromycin-atovaquone and <i>while receiving this treatment</i> , experienced relapse with fever; level of parasitemia increased from <1% to 30%
3	Atovaquone at 750 mg orally twice a day plus azithromycin at 250 mg orally once a day for 71 days	Became blood smear and PCR negative for <i>Babesia microti</i> ; experienced relapse with fever and positive blood smear (level of parasitemia, <1%) 11 weeks later	Atovaquone at 750 mg orally twice a day plus azithromycin at 250 mg orally once a day	After 51 days of azithromycin-atovaquone and <i>while receiving this treatment</i> , experienced relapse with an increase in the level of parasitemia from <1% to 7% and multiorgan failure

NOTE. PCR, polymerase chain reaction.

^a Of azithromycin-atovaquone.

of our patients failed to produce antibabesial IgG, and the third had only a low antibody level. The preponderance of data from animal systems indicates, however, that control of an established infection is dependent on an appropriate cellular immune response [14, 15]. Given that B cells also function as antigen-presenting cells for T cells and have other effects on T cell function, their depletion may affect cellular immune responses as well [16].

Our study has several potential limitations. The definition of drug resistance used was more stringent than generally accepted definitions of clinical resistance in patients treated for malaria [17]; thus, alternative definitions should be evaluated in future studies. We did not inoculate any of the *B. microti* strains into animals. Had this been done, it would have provided an opportunity to determine if such animal systems faithfully reproduce the clinical events that we observed. To date, however, testing for antimicrobial susceptibility in animal systems has not been standardized, and the results have not always been concordant between studies or with clinical experience [7, 8, 10–13]. We also did not attempt to elucidate the molecular basis for drug resistance, an important topic for future investigation.

In summary, we have found convincing clinical evidence that *B. microti* may become resistant to azithromycin-atovaquone during treatment of babesiosis with this combined drug regimen. Drug resistance is likely to develop only in highly immunocompromised patients and is unlikely to become a major public health problem as with malaria [18], because the reservoirs for this infection are rodents rather than humans, and babesia strains in rodents are not exposed to drug therapies. Although research is needed to determine the optimal therapy for highly immunocompromised patients with babesiosis, reducing the level of immunosuppression when possible would appear to be a desirable strategy.

Acknowledgments

We thank Barbara Keane, the technologist who first diagnosed babesiosis in patient 1, and Dr Raymond Dattwyler, Lisa Giarratano, Lenise Banwarie, Dr Julie Joseph, Dr Roshan Patel, Dr Cecilia Morales, Dr Louis Weiss, and Dr Lindita N. Coku for their assistance.

Financial support. P.J.K. is supported in part by a generous gift from the Gordon and Llura Gund Foundation.

Potential conflicts of interest. All authors: no conflicts.

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