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ID TEACHING CASES

Relapsed *Babesia microti* Infection Following Allogeneic Hematopoietic Cell Transplantation in a Patient With B-cell Acute Lymphoblastic Leukemia: Case Report and Review of the Literature

Joshua Rosenblatt,[1] Annie Leung,[4,5] Emily Baneman,[1] Risa Fuller,[1] Sarah Taimur,[1] Alberto E. Paniz-Mondolfi,[2] Adriana K. Malone,[3] Laura Kirkman,[4,5] and Samantha E. Jacobs[1]

[1]Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA, [2]Clinical Microbiology Laboratory, Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA, [3]Tisch Cancer Institute, Division of Hematology/Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, New York, USA, [4]Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York, New York, USA, and [5]Department of Microbiology and Immunology, Weill Cornell Medicine, New York, New York, USA

A patient with relapsed/refractory B-cell acute lymphoblastic leukemia developed babesiosis before allogeneic hematopoietic cell transplantation while on atovaquone for *Pneumocystis jirovecii* pneumonia prophylaxis. Despite receiving a prolonged course of atovaquone and azithromycin until whole-blood *Babesia microti* DNA was no longer detected by polymerase chain reaction, her post-transplant course was complicated by relapsed babesiosis. We investigate the potential host and parasite characteristics causing relapsing/persistent infection.

Keywords. *Babesia microti*; babesiosis; hematopoietic cell transplantation; immunocompromised host; tick-borne pathogens.

CASE REPORT

A 64-year-old female resident of Rockland County, New York, with relapsed/refractory B-cell acute lymphoblastic leukemia (ALL) awaiting hematopoietic cell transplantation (HCT) presented to the emergency department on 7/16/19 with fever and malaise for 1 day.

She had been previously diagnosed with CD20-positive Philadelphia chromosome-negative B-cell ALL in July 2018 and initiated treatment according to the dose-adjusted CALGB 10403 protocol [1]. Rituximab was incorporated into her regimen in the setting of CD20 positivity. Due to persistent minimal residual disease, she was transitioned to blinatumomab on 12/12/18 [2], then to salvage chemoimmunotherapy with inotuzumab plus mini–cyclophosphamide, vincristine, and dexamethasone (mini-CVD) on 4/9/19 [3]. She had been taking atovaquone 1500 mg daily for *Pneumocystis* prophylaxis since September 2018.

On hospital day 3, she was diagnosed with babesiosis after a Giemsa-stained thin blood smear revealed intra-erythrocytic and extracellular ring forms consistent with *Babesia microti* (2.5% parasitemia). The whole-blood *B. microti* polymerase chain reaction (PCR) and serum *B. microti* immunoglobulin M/immunoglobulin G (IgM/IgG; LabCorp Laboratories, Burlington, NC, USA) were positive and negative, respectively. The buffy coat smear was negative for *Anasplasma*/*Ehrlichia*. The source of infection was presumed tick-borne given the season and geography. Transfusion-transmitted infection was considered because the patient had received 2 red blood cell (RBC) transfusions in the preceding 5 weeks, but thought unlikely since the New York State blood supply is screened year-round for *Babesia* by nucleic acid amplification [4]. Figure 1 illustrates pertinent clinical and laboratory data (haptoglobin, percent parasitemia, and *B. microti* PCR positivity), as well as treatment regimens at the time of initial diagnosis and throughout the course of infection.

Because of her overall clinical stability, she was started on first-line therapy for babesiosis with azithromycin 1 g per os (PO) daily and atovaquone 750 mg twice daily on 7/18/19. Had she presented with more severe illness, alternative therapy might have been considered upfront given that she had previously been on atovaquone. Due to persistent fevers, clindamycin 600 mg IV every 8 hours was added on 7/21/19. She was transfused 1 unit of packed RBCs on hospital days 5 and 9. Her fevers eventually resolved, and the markers of hemolysis began to improve.

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Correspondence: Joshua Rosenblatt, MD, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, Box 1090, New York, NY 10029 (joshua.rosenblatt@mssm.edu).

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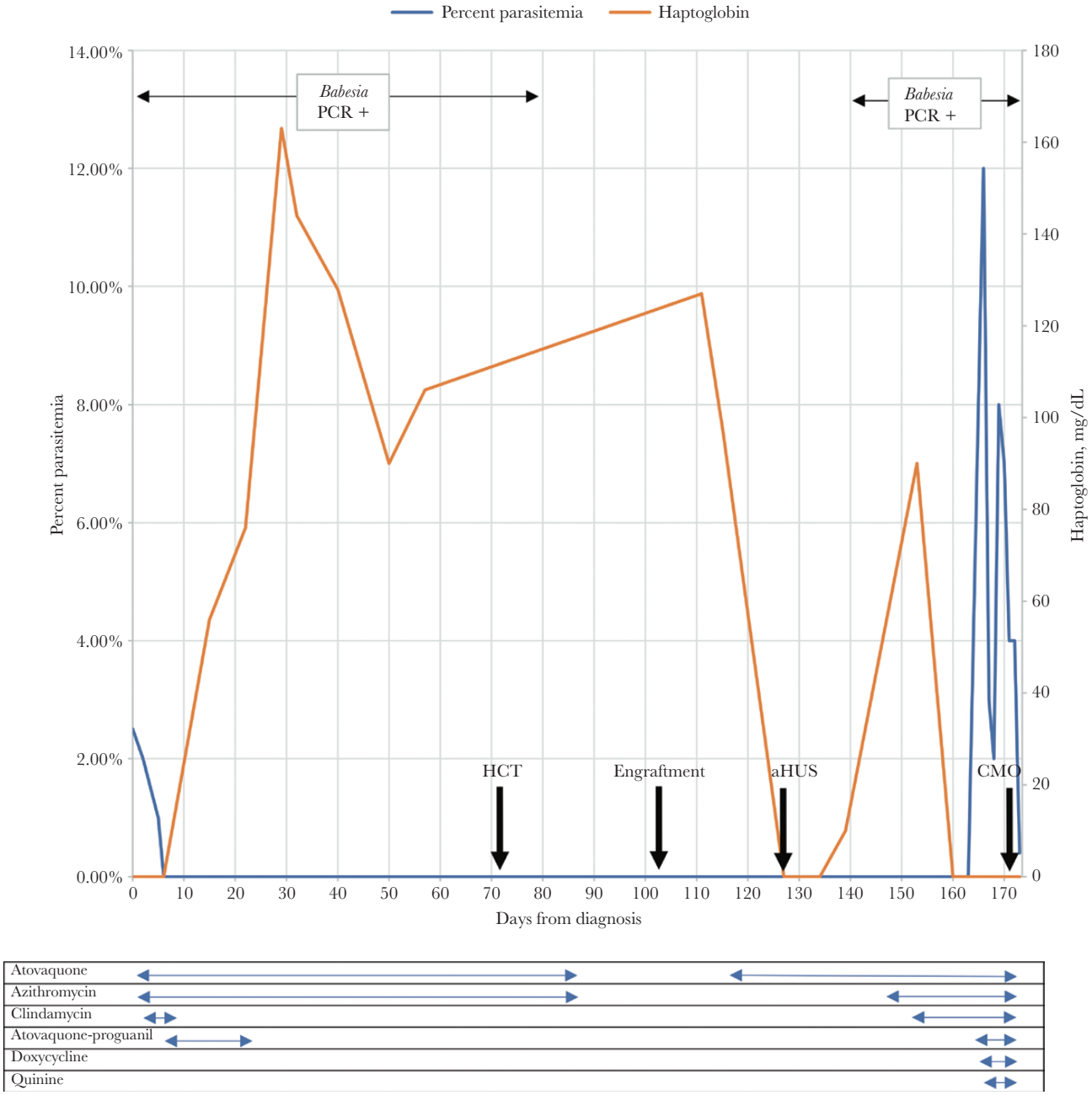

Figure 1. Illustration of case patient's clinical course, laboratory findings, and antibabesial therapies received from initial diagnosis to death. Abbreviations: aHUS, atypical hemolytic uremic syndrome; CMO, comfort measures only, HCT, hematopoietic cell transplantation; PCR, polymerase chain reaction.

She was discharged on 7/25/19 with atovaquone 750 mg twice daily, azithromycin 1 g PO daily, and atovaquone-proguanil 1 tablet daily with continued weekly monitoring of the parasite blood smear, *B. microti* PCR, and hemolysis labs [5–8, 13]. Although the haptoglobin normalized and the blood smear turned negative on 8/2/19, the *B. microti* PCR and IgM/IgG remained positive and negative, respectively. Atovaquone and azithromycin were continued, but atovaquone-proguanil was stopped. Allogeneic HCT, initially scheduled for mid-August, was delayed until the following month.

She was admitted on 9/20/19 for HCT. The reduced intensity conditioning regimen consisted of cyclophosphamide, fludarabine, and total body irradiation (200 cGy). She underwent haploidentical allogeneic HCT on 9/27/19 (71 days from diagnosis of babesiosis). Tacrolimus and mycophenolate mofetil were administered for prevention of graft-vs-host disease (GvHD). The immediate post-transplant course was complicated by delayed engraftment and neutropenic fever due to central line–associated *Staphylococcus epidermidis* and *Escherichia coli* bloodstream infection.

While admitted for HCT, the *B. microti* PCR became undetectable on 10/5/19 (79 days from diagnosis). Atovaquone and azithromycin were discontinued on 10/13/19 following 2 consecutive negative PCRs and in the setting of elevated liver function tests that were thought to be medication-related. She continued weekly *B. microti* PCR surveillance. Neutrophil engraftment occurred on 10/28/19, and she was discharged on 11/4/19 on tacrolimus for GvHD prophylaxis and acyclovir, trimethoprim-sulfamethoxazole (TMP-SMX), and voriconazole for antimicrobial prophylaxis.

She was readmitted on 11/11/19 with acute kidney injury, ultimately determined to be prerenal in etiology, but TMP-SMX was changed back to atovaquone 1500 mg daily for possible drug-induced renal toxicity. The hospital course was further complicated by recurrent hemolysis. The blood parasite smear and *B. microti* PCR remained negative, so she was administered the first of 4 weekly doses of eculizumab 900 mg for presumed tacrolimus-associated thrombotic microangiopathy on 11/25/19. Due to difficulty achieving therapeutic levels of sirolimus, she was changed to ruxolitinib 10 mg twice daily and prednisone 35 mg (0.5 mg/kg) daily for GvHD prophylaxis. She was discharged home on 11/27/19.

She was readmitted 8 days later on 12/5/19 with sepsis secondary to *E. coli* bacteremia from spontaneous bacterial peritonitis in the setting of noncirrhotic portal hypertension and worsening cholestasis. She was diagnosed with severe sinusoidal obstruction syndrome, formerly known as hepatic veno-occlusive disease, via liver biopsy and started on defibrotide on 12/11/19 with rapid, albeit partial, improvement in her hyperbilirubinemia.

During this hospitalization, the *B. microti* PCR turned positive on 12/6/19 (141 days from diagnosis), although no parasite forms were initially detected on blood smear. The *B. microti* IgM/IgG remained negative. She was continued on atovaquone, restarted on azithromycin 500 mg PO daily on 12/13/19, and later initiated on clindamycin 600 mg IV every 8 hours on 12/17/19 in the setting of fevers. While on this regimen, she developed high-grade parasitemia (10%–12%) on 12/31/19 (Supplementary Figure 1). Although the possibility of reinfection was explored, relapse was considered to be more likely given the clinical history. Despite our patient residing in an area endemic for *Ixodes scapularis*, there was a very limited window for possible reinfection. Following the first negative *B. microti* PCR on 10/5/2019, she was out of the hospital for only 13 days during November and December. Furthermore, there are considerably fewer cases this time of year [9].

Atovaquone-proguanil 4 tablets daily, doxycycline 100 mg PO every 12 hours, and quinine 648 mg every 8 hours were added on 1/1/20, 1/2/20, and 1/3/20, respectively. RBC exchange transfusion was considered, but ultimately deferred due to concerns about hemodynamic instability from significant fluid shifts. Shortly thereafter, despite the percent parasitemia decreasing to 0.4%, she developed worsening shock with multiorgan failure. After she and her family chose to pursue comfort care measures, she died on 1/12/20.

DISCUSSION

A history of B-cell lymphoid malignancy with either asplenia and/or treatment with immunosuppressive medications (rituximab, an anti-CD20 monoclonal antibody, in particular) is associated with more severe acute *Babesia* infection and poorer outcomes following standard treatment regimens [10]. The 2020 Infectious Diseases Society of America clinical practice guidelines recommend treating these patients for a longer duration—at least 6 weeks, including 2 weeks after parasites are no longer seen on blood smear [11].

Despite what is known about the clinical course and management of babesiosis in immunocompromised hosts, to our knowledge, there are only 3 other published case reports of babesial infections in HCT recipients (Table 1). These reports include a patient with refractory sickle cell anemia with transfusion-transmitted babesiosis in the immediate pretransplant period [12], a patient with chronic myelogenous leukemia who developed babesiosis 3 years post-transplant following treatment with rituximab and while on corticosteroids for chronic GvHD [13], and a patient with myelofibrosis who was diagnosed with babesiosis 14 months post-transplant after receiving tacrolimus, rituximab, and corticosteroids for GvHD [14]. Whereas the first patient responded to a 10-day course of therapy with atovaquone and azithromycin, the second patient had persistent parasitemia after 8 weeks on this regimen. It was not until he was transitioned to clindamycin and quinine, started on atovaquone-proguanil, and had azithromycin increased to 1000 mg daily that the parasitemia cleared. The third patient, meanwhile, was treated with doxycycline, clindamycin, and atovaquone for 6 weeks with rapid improvement in his hemolytic anemia.

Herein we report the fourth such case of babesiosis in a patient following HCT and, to our knowledge, the first and only case of a patient who underwent HCT while on antibabesial therapy with a positive *B. microti* PCR and a negative *B. microti* IgG, with a post-transplant course complicated by relapsed babesiosis. Of note, none of the previous case reports in HCT recipients comment on the *B. microti* IgG status at the time of initial diagnosis or following treatment. Adding to the uniqueness of this particular case, our patient suffered a relapse following 2 months of *B. microti* PCR negativity. In our review of the literature, only 1 other case of relapsed babesiosis following a negative *Babesia* PCR result has been described [6]. The patient, who had a remote history of Hodgkin's disease treated with splenectomy and chemoradiation, received 10 weeks of atovaquone and azithromycin until the *Babesia* PCR turned negative, but unfortunately developed relapsing infection

Table 1. Description of Published Cases of Babesiosis in Hematopoietic Cell Transplant Recipients

	Underlying Disease	Transplant Type	Immunosuppression at the Time of Initial *Babesia* Diagnosis	Time From HCT to Diagnosis	Mode of Diagnosis	Initial Treatment and Duration	Persistent/ Relapsing Infection	Subsequent Treatment	Final Outcome (Clinical and Microbiological)
Patient 1 [12]	Sickle cell anemia	Nonmyeloablative peripheral blood SCT	Unknown	~6 mo	Positive blood parasite smear, *Babesia* IFA, and *Babesia* PCR	Atovaquone 750 mg twice daily and azithromycin 600 mg daily for 10 d	No	N/A	Survived No further evidence of parasitemia by blood smear or *Babesia* PCR
Patient 2 [13]	CML	Allogeneic SCT	Prednisone 5–10 mg daily, MMF until 5 mo before diagnosis, and rituximab until 7 mo before diagnosis	3 y	Positive blood parasite smear	Atovaquone 750 mg twice daily and azithromycin 500 mg daily for 8 wk	Yes Persistent parasitemia and worsening hemolysis	Clindamycin 600 mg Q8H, quinine 628 mg Q8H, azithromycin 1000 mg daily, and atovaquone-proguanil (500/100 mg for the first 7 d, followed by 250/100 mg) twice daily for 6 wk, then azithromycin and atovaquone-proguanil alone	Survived Parasitemia became undetectable on 4-drug therapy; *Babesia* PCR turned negative the following month
Patient 3 [14]	Myelofibrosis	HLA-matched unrelated donor allogeneic HCT	Corticosteroids, tacrolimus, and rituximab	14 mo	Positive blood parasite smear and *Babesia* PCR	Doxycycline, clindamycin, and atovaquone for 6 wk	No	N/A	Survived Parasitemia resolved within 2 wk
Patient 4 (current case)	B-cell ALL	HLA-matched related donor allogeneic HCT	Rituximab until 9 mo before diagnosis	2 mo before HCT	Positive blood parasite smear and *B. microti* PCR	Atovaquone 750 mg twice daily and azithromycin (1000 mg initially, then 500 mg) daily for nearly 3 mo	Yes Relapsed infection with recurrent parasitemia and hemolysis	Atovaquone 750 mg twice daily, azithromycin 500 mg daily, clindamycin 600 mg Q8H, atovaquone-proguanil 4 tablets daily, doxycycline 100 mg Q12H, and quinine 648 mg Q8H	Expired

Abbreviations: ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; GvHD, graft-vs-host disease; HCT, hematopoietic cell transplantation; IFA, indirect fluorescent assay; MMF, mycophenolate mofetil; PCR, polymerase chain reaction; SCT, stem cell transplantation.

2 months later and ultimately died. Similar to our patient, she never had documented seroconversion during follow-up.

Our patient had several risk factors for relapsing/persistent infection, the most important of which was likely her net state of immunosuppression. Before infection, she had been treated with rituximab for her leukemia. Although the last dose had been administered ~9 months before her initial *Babesia* diagnosis, the immunomodulatory effects of rituximab have the potential to last >18 months [7]. Even after clearing her blood smear, she was unable to mount an antibody response to the parasite, as evidenced by persistently negative *B. microti* serologies, suggesting ongoing humoral deficiency.

Although the specific role of humoral immunity in achieving clinical cure remains unclear, it would appear that B cells, whether by producing antibodies and/or serving as antigen-presenting cells for T cells, are highly important, if not necessary, for sustained remission of babesiosis [7, 10]. Following a prolonged treatment course, our patient never seroconverted, possibly signifying incomplete parasite clearance despite a negative blood parasite smear and *B. microti* PCR.

Aside from chemotherapy for her ALL, our patient received multiple other immunosuppressive medications, including fludarabine (associated with profound and prolonged T lymphopenia) [15], eculizumab (associated with terminal complement deficiency) [16], and ruxolitinib (associated with impaired dendritic cell function and T-cell priming) [17]. Although none of these drugs have been implicated in relapsing/persistent babesiosis, their precise contribution (alone or in combination) remains unknown. Our patient also received an extended course of corticosteroids, which only further contributed to her degree of immunosuppression.

Based on prior reports, we also considered whether atovaquone or azithromycin drug resistance contributed to our patient's poor outcome. She was on atovaquone monotherapy during the initial incubation period and received nearly

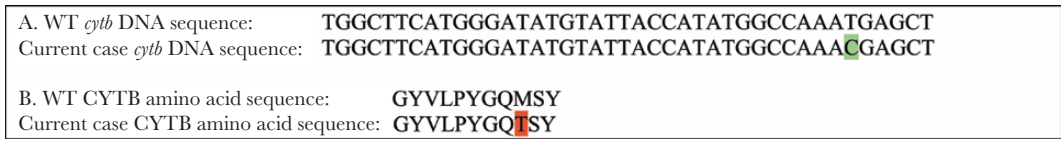

Figure 2. Possible molecular mechanism for atovaquone resistance. A, Comparison of wild-type and our case patient's c*ytb* genome sequence following relapse reveals a point mutation, highlighted in green. B. Comparison of wild-type and our case patient's CYTb amino acid sequence following relapse reveals a missense mutation with an amino acid substitution at position 134, highlighted in red, corresponding to possible resistance.

3 months of dual therapy following diagnosis. This prolonged antimicrobial pressure, coupled with incomplete parasite clearance due to profound immunosuppression, might have led to decreased susceptibility to 1 or both of these agents.

Stemming from findings in hamsters, there has long been concern about the risk of acquiring atovaquone resistance in the setting of monotherapy [18]. Further investigation has identified *Babesia* variants associated with relapsing disease in humans, although we still do not fully understand the clinical implications of these mutations [19]. A recent case report demonstrated a possible molecular mechanism of acquired resistance in a patient with chronic lymphocytic leukemia who developed resistance to both atovaquone and azithromycin following an initial 6-week course of standard therapy [8]. In the case of both drugs, resistance was due to point mutations in the genes encoding their respective target proteins, with concomitant predicted changes in their binding sites.

Using the methods described in the Supplementary Data, PCR was performed using the primers listed in Supplementary Table 1 on genomic samples extracted from archived thin blood smears collected at the time of our patient's babesiosis relapse. PCR analysis revealed a point mutation in the *cytb* gene, which encodes the target for atovaquone (Figure 2). This mutation (ATG to ACG) alters the amino acid at position 134 (M134T) in the highly conserved CYTb ubiquinol-binding pocket (Q_O domain). Mutations at this position have been previously described in cases of resistance to atovaquone in both babesiosis and malaria [20, 21]. Although there was insufficient specimen volume to amplify the *cytb* gene from the pretreatment sample, we suspect that this mutation arose de novo at some point during our patient's extended treatment course. Despite extensive exposure to azithromycin, PCR on these same samples demonstrated a wild-type sequence for *rpl4*, the gene encoding the target for azithromycin.

In summary, the management of babesiosis in HCT patients in the peritransplant period remains a significant challenge. These patients are at significantly increased risk of persistent/relapsing infection due to profound and prolonged immunosuppression. Complicating matters are that reduction of immunosuppression increases the risk of GvHD, extended antibabesial therapy carries a risk of acquired drug resistance, and no standardized approach to monitoring these patients has been established.

Taking all this into account, it may be most prudent to consider continuation of antibabesial therapy in highly immunocompromised hosts until it is possible to achieve some degree of immune reconstitution, either through reduction of iatrogenic immunosuppression or remission of the underlying immunosuppressive condition. Further studies are needed in this population to help define the most appropriate combination of medications, the optimal duration of therapy, and the best approach to monitoring. Given the rapidly expanding population of immunosuppressed hosts, in part due to novel therapeutics compromising humoral and cell-mediated immunity, this research would be especially timely.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Patient consent. Consent was unable to be obtained from the patient before death. Ethical board approval was not believed to be indicated because this case report did not involve human subjects research.

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