Case Report: Delayed Hemolysis after Parenteral Artesunate Therapy for Severe Malaria in Two Returning Canadian Travelers

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Abstract. Delayed hemolysis after parenteral artesunate has been described in Europe and Asia, but until recently had not been reported in patients receiving the artesunate product used in the United States and Canada. We report two cases of severe delayed hemolysis after the treatment with intravenous artesunate in Canada.

BACKGROUND

Artesunate reduces mortality from severe malaria in adults and children with fewer adverse effects compared with the previous standard of intravenous quinine.^{1,2} Since 2010, the World Health Organization has recommended artemisinins as first-line therapy for severe malaria.³ Parenteral artesunate is available through the Canadian Malaria Network (CMN) with supplies positioned in multiple centers across the country. The artesunate supplied to CMN and the Centers for Disease Control and Prevention (CDC) is produced under good manufacturing practice, and obtained from the Walter Reed Army Institute of Research, Silver Spring, MD. Until recently, this drug class had appeared remarkably free of serious adverse effects. Recent case series from Europe, Asia, and subsequently Africa describe patients treated with intravenous artesunate products who experienced post-artemisinin delayed hemolysis (PADH).^{4–12} Until recently, there had been no reports of this complication among patients treated with parenteral artesunate in North America.^{13–15}

According to a recently proposed definition, PADH is characterized by a decrease of $\geq 10\%$ hemoglobin level in the setting of a haptoglobin level < 0.1 g/L and an increase of lactate dehydrogenase (LDH) > 390 U/L at least 7 days after initiation of parenteral artesunate.¹⁴

CASE 1

In December 2013, a 44-year-old male presented to the emergency department, University of Alberta Hospital in Edmonton, Alberta, Canada, with fever and confusion, 4 days after returning from 1 month working in Cameroon. He had taken no malaria chemoprophylaxis or treatment, and specifically, had not received any artemisinin derivative. His only medical history was of treated hypertension; chronic hepatitis C infection was diagnosed during this hospitalization. On examination, he was hypotensive, febrile to 40°C , and markedly jaundiced. At presentation, his hemoglobin level was 164 g/L (6% nucleated red blood cells [RBCs]), white blood cell count $17.3 \times 10^9/\text{L}$, and platelets $4 \times 10^9/\text{L}$. *Plasmodium falciparum* trophozoites with schizonts were observed in the peripheral blood smear with a parasitemia

of 12.3%. Speciation was confirmed by real-time polymerase chain reaction.¹⁶ He required immediate intubation and ventilation for a depressed level of consciousness. Intravenous artesunate at 2.4 mg/kg/dose was administered at 0, 12, 24, and 48 hours post admission (total: 9.6 mg/kg). On day 3, thin and thick smears were negative. Oral atovaquone 1 g/proguanil 400 mg was given daily for 3 days. Acute kidney injury required continuous renal replacement therapy for 4 days, followed by a single dialysis 3 days later.

His hemoglobin level began to drop immediately after admission (Figure 1); increasing red cell transfusions were required after a nadir of 68 g/L was reached on day 15. Hemolysis markers—a presenting total bilirubin of 634 $\mu\text{mol/L}$ and LDH peaking at 1,369 U/L—were present immediately, consistent with acute malaria. On day 2, the haptoglobin was undetectable. The haptoglobin became detectable on day 8 of admission, but again dropped below the level of detection (< 0.10 g/L, normal 0.30–2.00 g/L). On day 25, the patient met all criteria for PADH with a hemoglobin level of 78 g/L, undetectable haptoglobin, a reticulocyte percentage of 15.7%, and LDH level 426 U/L. Between days 15 and 28, the patient received 11 units of packed RBCs before hematological recovery; by day 47 his hemoglobin level was up to 98 g/L, and had finally normalized at 141 g/L at 3.5 months post presentation.

No other explanation was found for his anemia. At no time were the blood film findings consistent with a microangiopathic or autoimmune hemolytic process. Coagulation parameters were not suggestive of disseminated intravascular coagulation. Blood loss studies, including colonoscopy and abdominal computed tomography, were negative. Vitamin B₁₂, folate, and iron levels were normal. The direct antiglobulin test became weakly positive on January 13 (immunoglobulin G positive, complement negative), but the eluate was nonspecific. All clinically relevant antibodies (anti-D, C, c, E, e, Cw, K, k, Fya, Fyb, Jka, Jkb, M, N, S, and s) were excluded from both plasma and eluate by the reference transfusion laboratory of northern Alberta, and all antibody screens remained negative.

CASE 2

In September 2014, a previously healthy 55-year-old man presented to the emergency department at the University of Alberta Hospital with fever, nausea, and vomiting 5 days after returning from the Democratic Republic of Congo, where he had been working for the previous 5 weeks. He had not been taking malaria prophylaxis. On assessment, he was confused, febrile at 38.8°C , and tachycardic with a blood pressure of

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FIGURE 1. Hemoglobin and lactate dehydrogenase (LDH) trends for patients 1 and 2. Arrows indicate transfusions and number of packed cells.

85/46 mmHg, and had tender hepatomegaly. His malaria diagnosis was delayed for several hours by the laboratory protocols in place for possible Ebola. Laboratory investigation revealed an anemia of 105 g/L, thrombocytopenia at $19 \times 10^9/L$, leucopenia of $3.7 \times 10^9/L$, and a lactate of 7.9 mmol/L. His bilirubin peaked at 58 $\mu\text{mol/L}$ with an LDH of 878 U/L and a *P. falciparum* parasitemia of 35%. Intravenous artesunate therapy, 2.4 mg/kg/dose, was given at 0, 12, 24, and 48 hours post admission. His intensive care unit stay was uncomplicated and the parasitemia had cleared 72 hours post artesunate initiation. Treatment was completed with 3 days of oral atovaquone 1 g/proguanil 400 mg and the patient was discharged home on day 6. His last hemoglobin measurement taken in hospital was 77 g/L (Figure 1), with his bilirubin improved to 11 $\mu\text{mol/L}$ and LDH to 390 U/L. No RBC transfusions were administered during his hospital stay.

Fifteen days post artesunate initiation, he presented to the emergency department with increasing weakness and was found to have a hemoglobin level of 59 g/L with normal platelets of $161 \times 10^9/L$ and total white blood cell count of $6.2 \times 10^9/L$. His haptoglobin was < 0.1 g/L, LDH increased to 1,015 U/L, and bilirubin to 40 $\mu\text{mol/L}$ with a reticulocyte percentage up to 12.9% (Figure 1). As in the first case, no other explanation was found for his acute anemia with any history of bleeding, negative direct antiglobulin test, normal vitamin B₁₂ level, and normal thyroid, liver, and kidney function. There were no peripheral blood smear findings suggestive of a microangiopathic or autoimmune hemolytic process, nor any further malarial parasites. He required 2 units of packed RBCs. His bilirubin and haptoglobin normalized 20 days post artesunate treatment.

Both of these presumed serious drug adverse effects were reported to the Canadian Malaria Network.

DISCUSSION

Severe malaria is uncommon in Canada, with an average of 14 imported cases per year¹⁷; artesunate is the drug of choice in these patients. Anemia is one of the most important complications of malaria, primarily among children in endemic areas. Multiple mechanisms contribute to malarial anemia including direct and indirect effects on both parasitized and noninfected erythrocytes, mediated in part by malarial proteins and host immune response as well as enhanced splenic activity and decreased or ineffective erythropoiesis.¹⁸ Cases of artemisinin-related hemolysis may not have been recognized in the past because they were attributed to these relatively common, established mechanisms of anemia. The degree and timing of hemolytic anemia described in the case series from Europe and in our patients lie outside the range of previous experience with malaria treatment. In uncomplicated malaria, the hemoglobin nadir is usually around 1 week after treatment initiation with a rapid recovery to normal over the subsequent 3–4 weeks.¹⁹

In our first patient, hospital discharge was delayed since the concept PADH was not recognized and the patient underwent invasive procedures in an attempt to explain his anemia. Previously, it was suggested that the absence of cases of PADH reported from North America might be explained by the differences between the artesunate products used in North America and Europe.¹³ Aside from the two cases reported here, one retrospective and one prospectively identified

case that meet the case definition for PADH, were recently identified by the CDC¹⁴ and one further case was reported from Canada.¹⁵ These cases would appear to refute the hypothesis that PADH is attributable to a specific drug formulation. With the introduction of more active surveillance on the part of CDC, the lower number of reports of PADH in North America relative to Europe remains incompletely explained. In the previously reported non-North American cases of PADH, most patients had high parasitemias and had been treated with higher doses of intravenous artesunate (8–20 mg/kg).

Recent work has provided a likely mechanism contributing to PADH. In contrast with other antimalarials, artemisinins kill and expel the parasites without destroying the erythrocyte. These altered, “pitted,” erythrocytes remain in circulation. The delayed, synchronous destruction of this population of pitted erythrocytes accounts for the delayed hemolysis.²⁰ Hemolysis with exposure of RBCs to high doses of several artemisinin derivatives has also been described in vitro.²¹

CONCLUSION

Accumulating evidence supports an association between intravenous artesunate (and possibly other artemisinin derivatives)²² and delayed hemolysis. All patients receiving intravenous artesunate should have systematic follow-up of hemoglobin levels up to 4 weeks post treatment. This will enable recognition of patients with delayed onset anemia and better define the frequency and characteristics of this phenomenon, particularly in North America.

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