

**Title: Blackwater fever in a non-immune patient with *Plasmodium falciparum* malaria after intravenous artesunate**

**Running title: Blackwater fever after artesunate**

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## **Abstract**

Blackwater fever was typically reported after quinine administration, although it is poorly recognized in patients receiving artesunate. This case describes a blackwater fever in a non-immune patient after artesunate for severe malaria. It highlights the importance of monitoring hemolytic parameters in severe malaria to avoid renal impairment or severe anaemia.

## Background

Blackwater fever is defined as an ~~early acute~~ intravascular haemolysis associated with marked haemoglobinuria and occasionally renal injury, jaundice and fever in patients with malaria. It was a condition typically reported after ~~the administration of a few doses of~~ quinine ~~administration~~ for malaria treatment in non-immune patients living in Africa, ~~classically associated with low parasitemia~~ [1]; ~~but~~ it has been also described in immune population [2]. With the introduction of other drugs but quinine in the treatment of malaria, the incidence of blackwater fever has decreased [3]. It occurred in less than 10% of the participants in the artesunate arm in AQUAMAT and SEAQUAMAT studies, but without significant differences with quinine [4,5]. ~~However~~ ~~A different scenario is;~~ delayed haemolysis associated to artesunate, ~~usually presented between day 7 and day 21 after the start of the treatment; it~~ has been described in up to 25% of cases in some studies [6]. In this cases, haemolysis is probably caused by a delayed clearance of pitting erythrocytes [6]. ~~This~~

~~The present~~ case report describes a blackwater fever syndrome in a non-immune patient early after treatment with intravenous artesunate for severe *Plasmodium falciparum* malaria.

## Case presentation

A 57 years old Spanish male presented to a regional hospital in Spain with a 3 day history of fever, chills, sweating, headache and generalized arthralgia and myalgia after a trip to Guinea Conakri 10 days before symptoms started (September 2016).

The patient had stayed in Conakri city for six months. He never took malaria prophylaxis and he did not travelled to rural areas. As a medical record the patient had

suffered from right retinal detachment associated to lens dislocation 18 years ago and had cervical hernias (C4-C7) without neurological complications. He was not taking any chronic medication and he did not have any allergies. No previous malaria history was recorded.

He was admitted under malaria suspicion. A thick blood smear revealed a 4% *Plasmodium falciparum* parasite count. After diagnosis, the patient was referred to the National Reference Centre, Hospital Clinic of Barcelona, during the same day. At the time of admission he did not present with any other conditions defining severe malaria than the parasite count above 2.5% in a non-immune patient. On examination the patient was stable and the physical examination was normal with the exception of slight mental confusion. The patient was admitted to an intermediate care unit and intravenous artesunate was started. After the second dose (180 mg = 2.4 mg per patient kilogram per dose), 12 hours after the first one, the patient started with intense coluria (coke-like color) (**Figure 1**). Urine analyses showed no red cells but haemoglobinuria. At that time parasitemia was 0, and parameters of haemolysis appeared, with increased levels of bilirubin and lactate dehydrogenase (peak day 2), and reduced levels of hemoglobin and haptoglobin (Table 1). Thus the diagnosis of intravascular haemolysis with haemoglobinuria (or blackwater fever) was established. Other causes or triggers of the haemolysis than artesunate were ruled out: serologic tests for dengue and leptospira as well as blood cultures and direct Coombs test were negative; there was no evidence of thalassemia, he had normal glucose-6 phosphate dehydrogenase activity and he did not have past history of splenectomy. Afterwards, intravenous artesunate was stopped and the treatment was switched to atovaquone-proguanil (250 mg atovaquone/100mg proguanil per tablet, 4 tablets per day) for 3 more days without complications and progressive clinical recovery. The patient did not need blood transfusions or suffered of

renal impairment. Two days after the beginning of the hemolytic event, clinical pneumonia, without final microbiological confirmation diagnosis, was diagnosed with clinical and radiological criteria, and the patient received ceftriaxone for 5 days and levofloxacin for 10 days. Haemoglobinuria disappeared by day 5 of admission with slow recovery of hemolytic parameters. The patient was discharged after 7 days of admission.

The patient was completely recovered after four weeks and no signs of delayed hemolysis were observed.

### **Discussion and Conclusions**

Herein we have described the case of a patient with hemolytic anemia and hemoglobinuria in the context of malaria under treatment with artesunate (blackwater fever). The acute haemolysis in our case could be due to malaria itself. Plasmodium can induce haemolytic anaemia due to the inflammatory cascade added to the rupture of parasitized erythrocytes, parasitized or not[7]. However, the fact that the hemolysis started after two doses of artesunate makes the drug suspicious of a relevant role in its development. Artesunate can induce oxidant haemolysis, although in lesser degree than quinine or mefloquine [8]. Artesunate also changes the erythrocyte membrane increasing its fragility [8]. Accordingly, artesunate has been recently related to acute haemolysis with haemoglobinuria in a Plasmodium knowlesi Malayan patient[9] as well as a Cambodian patient after artesunate derivatives in an uncomplicated Plasmodium falciparum infection[190], besides the previously commented description in larger studies amongst immune population in the past (AQUAMAT[4] and SEAQUAMAT[5]).

Patients with splenectomy, thalassemia or low glucose-6 phosphate dehydrogenase activity have more risk to suffer severe haemolysis during Plasmodium infection<sup>[10]</sup>, although our patient did not have any known underlying condition. Other described triggers of haemolysis that our patient presented ~~are high parasitemia, which is related with blackwater fever in murine models[11], and is~~ the presence of a bacterial infection<sup>[9]</sup>, although pneumonia was diagnosed after the beginning of the haemolysis.

The clinical consequences in the case described were mild but highlights the importance to monitor urine as well as hemolytic parameters in severe malaria after artesunate treatment, especially in splenectomized or thalassemic patients, in order to avoid renal impairment or severe anaemia.

### **Declarations**

This work is original and it has not been published elsewhere or it is currently under consideration for publication in other journals. All authors supervised the healthy status of the patient while admitted. All authors approved the manuscript and its submission and they have full access to the data and edition of the draft. We also do not have conflicts of interest to disclose and no sources of funding.

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<b>Days of admission/lab parameters</b>	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 8</b>
<b>Total Bilirrubin mg/dL</b>		4.7	3.1	3.1	1.3	
<b>Indirect bilirrubin mg/dL</b>		2.2	0.9	0.4	0.6	
<b>LDH U/L</b>	721	2359	2005	951	721	618
<b>Haemoglobin g/L</b>	155	147	147	125	128	118
<b>Hematocrit %</b>	43	43	41	36	35	36
<b>Haptoglobin g/L</b>		0.010	0.030			
<b>Haemoglobinuria (+)</b>		3		2		0

Table 1. Change in laboratory parameters during admission in Hospital Clinic of Barcelona. LDH: lactate dehydrogenase