MAJOR ARTICLE

# Delayed Hemolysis After Treatment With Parenteral Artesunate in African Children With Severe Malaria—A Double-center Prospective Study

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***Background.*** Parenteral artesunate is recommended as first-line therapy for severe malaria. While its efficacy is firmly established, data on safety are still incomplete. Delayed hemolysis has been described in hyperparasitemic nonimmune travelers, but it is unknown if African children are equally at risk.

***Methods.*** Children aged 6 to 120 months with severe malaria were followed up after treatment with parenteral artesunate in Lambaréné, Gabon, and Kumasi, Ghana. The primary outcome was incidence of delayed hemolysis on day 14.

***Results.*** In total, 72 children contributed complete data sets necessary for primary outcome assessment. Delayed hemolysis was detected in 5 children (7%), with 1 child reaching a nadir in hemoglobin of 2.8 g/dL. Patients with delayed hemolysis had higher parasite counts on admission (geometric mean parasite densities (GMPD) 306 968/μL vs 92 642/μL, $P = .028$) and were younger (median age: 24 months vs 43 months, $P = .046$) than the rest of the cohort. No correlation with sickle cell trait or glucose-6-phosphate-dehydrogenase deficiency was observed.

***Conclusions.*** Delayed hemolysis is a frequent and relevant complication in hyperparasitemic African children treated with parenteral artesunate for severe malaria. Physicians should be aware of this complication and consider prolonged follow-up.

***Clinical Trials Registration.*** Pan-African Clinical Trials Registry: PACTR201102000277177 (www.pactr.org).

***Keywords.*** Severe malaria; artesunate; adverse events; hemolysis; African children.

The optimal management of malaria is a global health priority. Between 700 000–1.2 million fatalities due to malaria in 2010 strongly supports deployment of antimalarial interventions that have proven efficacy [1, 2].

For the treatment of malaria, comparative trials of artesunate and quinine have established that in both adults and children lives can be saved with artesunate [3–6]. In addition to its antiparasitic superiority, artesunate was demonstrated in a meta-analysis to have a better short-term safety profile than quinine [5].

Data on adverse events after the treatment period, however, are scarce mainly because trials have not been designed to detect such effects. In SEAQUAMAT, follow-up was limited to the in-hospital stay with a median length of 5 days [3]. In AQUAMAT, only children who had not fully recovered at discharge were actively followed up after 28 days with a focus on assessment

Received 19 September 2013; accepted 17 December 2013; electronically published 26 December 2013.

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**The Journal of Infectious Diseases 2014;209:1921–8**



DOI: 10.1093/infdis/jit841

of neurological sequelae [4]. No standardized follow-up of laboratory parameters was performed and possible hematological side-effects may have gone unnoticed. Moreover, late post-discharge complications may remain undetected under routine hospital conditions due to the fragmentary health infrastructure in endemic countries [7]. Likely for these reasons, it was only after the introduction of parenteral artesunate as first line treatment of severe imported malaria in Europe that initial reports described delayed hemolysis after parenteral treatment with artesunate [8–11]. Clinically relevant delayed hemolysis appeared 2–4 weeks after acute malaria and resulted in the need of repeated transfusions in the most severely affected patients. A common risk factor was initial hyperparasitemia, although at the time of delayed hemolysis all parasites had been cleared.

The relevance and generalizability of these observations to malaria patients in endemic countries is not yet established. Because of its proven superiority in particular related to antiparasitic effectiveness and survival, parenteral artesunate is recommended as first-line therapy in severe malaria in endemic countries. In these areas, however, delayed hemolysis may be of particularly high relevance as in African children multiple additional causes of anemia such iron deficiency, hemoglobinopathies, malnutrition, and chronic infections exist [12]. To provide information on the clinical relevance of delayed hemolytic anemia in children with severe malaria treated with parenteral artesunate in Africa, we report results from a prospective observational study.

## PATIENTS AND METHODS

The study was conducted between April 2012 and September 2012 as a substudy of the "Comparative, Open Label, Dose and Regimen Optimization Follow-up Study of Intravenous and Intramuscular Artesunate in African Children with Severe Malaria" (Pan-African Clinical Trials Registry: PACTR201102000277177) and was implemented at the Centre de Recherches Médicales de Lambaréné, Gabon, and the Komfo Anokye Teaching Hospital in Kumasi, Ghana. Both sites are hyper- to holoendemic for malaria and transmission is perennial with some seasonal variation [13, 14].

### Ethics Statement

The study protocol was approved by the Institutional Review Board of the Centre de Recherches Médicales in Lambaréné, Gabon and by the Committee on Human Research Publication and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

### Patients

Children aged 6 months to 10 years with presence of asexual *P. falciparum* parasites (>5000 parasites/μL on the initial thick blood smear) and severe malaria according to definitions of the Severe Malaria in African Children (SAMC) network were included after written informed consent of the parent or legal guardian [15–17]. Children with known hypersensitivity or serious adverse reactions to artemisinins, children having participated in any investigational drug study during the 30 days prior to screening, and those having obtained adequate antimalarial treatment within 24 hours prior to admission were excluded from the study.

All patients were treated with a total of 12 mg/kg body weight of parenteral artesunate (Guilin Pharmaceutical Company, Shanghai, China) followed by a full course of weight-adapted artemether/lumefantrine. Artesunate was given according to 3 randomly allocated regimens (3 doses of 4 mg/kg body weight intravenously, 3 doses of 4 mg/kg body weight intramuscularly, or 5 doses of 2.4 mg/kg body weight intramuscularly). Supportive treatment and blood transfusions were administered as clinically indicated. All patients received folic acid supplementation for at least 14 days. The study was a follow-up trial of the recently published work showing that a simplified 3-dose artesunate regimen for severe malaria is equally efficacious as the conventional 5-dose regimen. Study procedures of the current trial followed those of the earlier trial [16].

### Methods

Clinical parameters, information on concomitant medication, current symptoms and blood samples were obtained at presentation (day 0) and at follow-up visits on days 7 (±2), 14 (±2), and 28 (±2). Parasite species and density were determined from Giemsa-stained thick blood films. Hematological and biochemistry assessments on days 7, 14, and 28 were added to the initial study protocol. Reticulocyte production index (RPI) as a marker of bone-marrow erythropoiesis was calculated as before [18]. RPI corrects the reticulocyte count for the degree of anemia. Serum samples were centrifuged and stored at −80°C until further analyses.

DNA for genotyping of red cell polymorphisms was obtained from thick blood smears of participants. Slides were treated with xylene to remove the immersion oil. Moistened blood spots were scraped off into microcentrifuge tubes and dissolved by pipetting. DNA was then extracted according to the DNA extraction kit's instructions (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). Genotyping for sickle cell disease and glucose-6-phosphate-deficiency was determined by high-throughput postamplification genotyping using fluorescent melting curve assays on 384-well microplate formats in a homogeneous system (LightTyper; Roche Diagnostics) as before [19, 20].

The primary endpoint was delayed hemolysis on day 14. Delayed hemolysis was defined as the coexistence of: (i) low haptoglobin (<0.30 mg/dL) on day 14, (ii) any decrease in hemoglobin (Hb) between days 7 and 14, and (iii) any increase in lactate dehydrogenase (LDH) between days 7 and 14 leading to a LDH level of over 350 U/L on day 14.

### Statistics

The sample size calculation was based on the assumption that the frequency of hemolysis on day 14 was 5% in the context of this study. Based on $\alpha = 0.05$ and a power of 0.9, 92 children with complete data sets (completing days 0, 7, and 14) would have to be included. Allowing for a lost to follow-up on day 14 of 10%, a mortality of severe malaria of 2.5% and a lack of complete laboratory data for the complex outcome definition we aimed to include 102 children. Statistical analysis was performed with the SPSS 17.0 software package (SPSS Inc, Chicago, IL). Demographic, clinical, and biochemical parameters were compared between study sites and between outcome groups applying Student *t* test or Mann–Whitney *U* test, as well as 2-tailed Fisher exact test for continuous parametric, nonparametric, and categorical variables, as appropriate.

## RESULTS

A total of 102 children were recruited. Two children died early after admission, with no further deaths during the study period. The median age of the patients was 37 months (interquartile range: 22–62), and 31 (30%) were female. Geometric mean parasite density (GMPD) was 125 769 parasites/μL. Table 1 shows the demographic and baseline laboratory parameters for the entire study population as well as separately for the 2 study sites. Prostration was found in 57 (56%), hyperparasitemia in 38 (37%), jaundice in 18 (18%), severe anemia in 14 (14%), and multiple convulsions in 11 children (11%).

Seventy-two children with complete sets of Hb and LDH values for days 7 and 14 and haptoglobin values on day 14 were available. In 5 (7%; 95% confidence interval [CI], 1%–13%) of these 72 children, the predefined signs of delayed hemolysis on day 14 were present. None of the patients with delayed hemolysis had detectable blood stage parasites on day 14. Figure 1 illustrates the time course of Hb and LDH levels of these 5 patients through the study period and shows a significantly lower Hb on day 14, as well as significantly higher LDH activity on day 7 and day 14 in patients with hemolysis. For 3 of these 5 hemolytic patients, data for day 28 were available. All 3 showed a tendency of normalization of Hb levels after 4 weeks, but Hb still had not returned to the reference range. One patient (H4) was lost to follow-up by day 28. Patient H5 presented with reappearance of prostration on day 14. The hemoglobin concentration was 2.8 g/dL, and the patient required repeated blood transfusions. After stabilization, the patient was transferred to the National Pediatric Oncology Unit in Ghana for further diagnostic work-up. A concomitant malignant disease was excluded as a cause for the massive hemolysis seen in this patient. Four months after admission to the trial, all laboratory values returned to normal ranges, and the patient was well. None of the other patients showed any significant clinical abnormalities on day 14, and no specific supportive treatment was prescribed.

Table 2 shows demographic and clinical parameters of the patients with delayed hemolysis. Only one of the patients was older than 2 years, and all had parasite levels >200 000 parasites/μL. In an exploratory approach, we looked for differences in baseline characteristics between patients presenting with and without delayed hemolysis on day 14. Patients with delayed hemolysis on day 14 showed higher initial parasite levels (GMPD 306 968 parasites/μL vs 92 642 parasites/μL, $P = .028$) and were younger (median age: 24 months vs 43 months, $P = .046$).

All patients who developed hemolysis on day 14 had a hemoglobin genotype AA, whereas in the patients without hemolysis 4 children (5%) had sickle cell trait (HbAS) and 5 children (8%) had hemoglobin C trait (HbAC). No sickle cell disease (HbSS)

**Table 1. Baseline Demographic and Routine Clinical Parameters**

| Characteristic | All | Lambaréné | Kumasi | *P* Value* |
|---|---|---|---|---|
| n (%) | 102 | 49 (48) | 53 (52) | . . . |
| Age in months, median (IQR) | 37 (22–62) | 49 (23–78) | 31 (22–48) | **.027** |
| Female gender, n (%) | 31 (30) | 15 (31) | 16 (30) | 1.000 |
| Temperature, °C | 38.3 (37.8–38.3) | 38.3 (38.0–38.6) | 37.8 (37.6–38.2) | **.032** |
| Geometric mean parasite density, /μL | 125 769 (95 846–165 032) | 71 826 (49 553–104 110) | 211 103 (148 895–299 329) | **<.001** |
| Hemoglobin, g/dL | 8.1 (7.6–8.5) | 9.0 (8.4–9.5) | 7.2 (6.6–7.9) | **<.001** |
| WBC, $\times 10^3$/μL | 10.1 (9.3–10.9) | 9.6 (8.5–10.6) | 10.7 (9.6–11.7) | .115 |
| Glucose, mmol/L | 4.8 (4.4–5.2) | 4.7 (4.2–5.3) | 4.9 (4.2–5.5) | .526 |
| Creatinine, μmol/L | 36 (26–47) | 31 (27–34) | 42 (21–62) | .317 |
| Total bilirubin, mg/dL | 1.4 (1.2–1.7) | 1.1 (0.8–1.4) | 1.7 (1.4–2.1) | **.010** |
| Blood transfusions, n (%) | 32 (31) | 7 (14) | 25 (47) | **<.001** |

Abbreviations: IQR, interquartile range; WBC, white blood cells.

Data are mean (95% confidence interval), unless otherwise specified.

*For comparisons between Lambaréné and Kumasi.

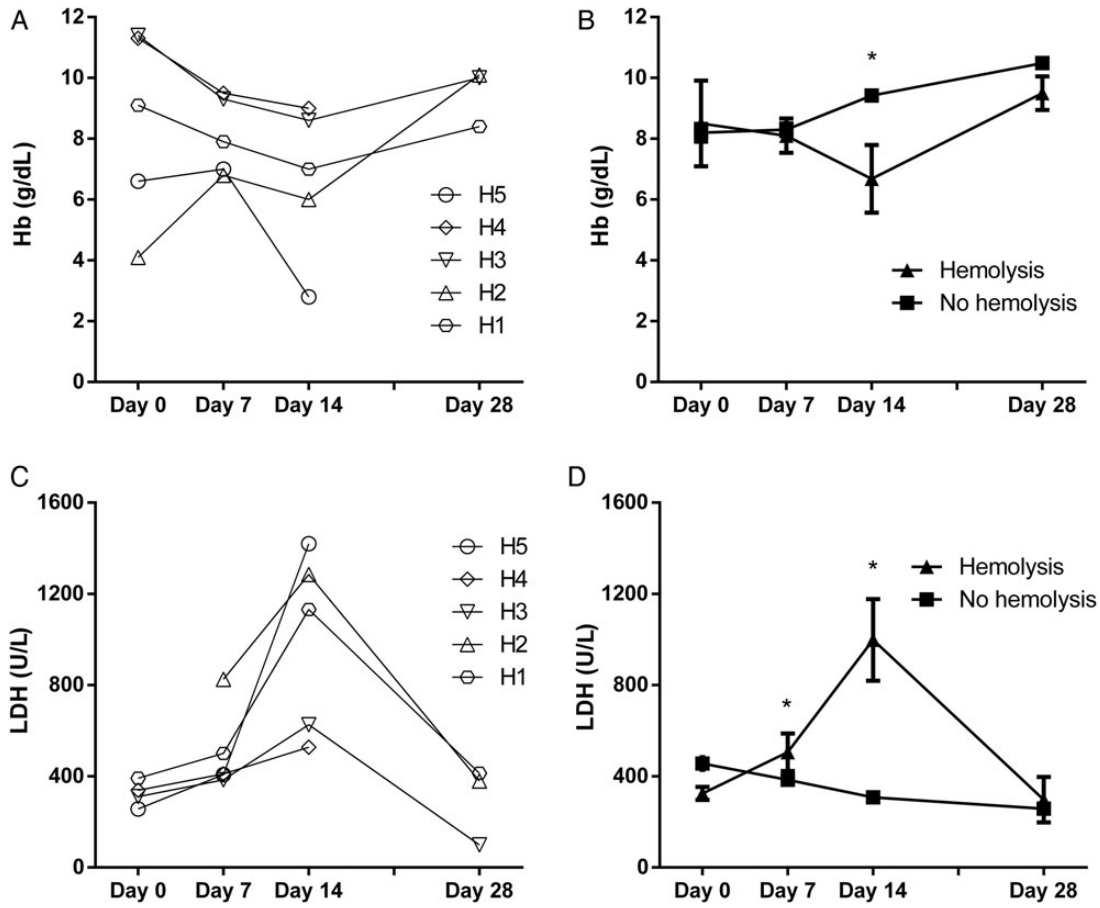


**Figure 1.** Time course of laboratory parameters of individuals with hemolysis. *A*, Hemoglobin levels over time in individual patients with hemolysis. *B*, Comparison of hemoglobin levels in patients with hemolysis vs those without hemolysis. *C*, Lactate dehydrogenase levels over time in individual patients with hemolysis. *D*, Comparison of lactate dehydrogenase levels in patients with hemolysis vs those without hemolysis. **P* < .05.

or HbC disease (HbCC) genotype was detected in any of the included children. All boys with hemolysis had a wild-type glucose-6-phosphate-dehydrogenase ($Gd^B$) genotype, whereas 1 girl was heteorozygous ($Gd^B/Gd^{A-}$) and 1 girl could only be typed for exon 4 but the presence of a $Gd^{A-}$ allele could thereby be excluded.

**Table 2. Baseline Characteristics of Patients With Signs of Hemolysis Compared to Patients Without Hemolysis on Day 14**

| | No Hemolysis on Day 14 | Hemolysis on Day 14 | *P* Value |
|---|---|---|---|
| N | 67 | 5 | |
| Age in months, median (IQR) | 43 (24–78) | 24 (9–43) | **.046** |
| Female gender, n (%) | 21 (31) | 2 (40) | .652 |
| GMPD, /µL | 92 642 (66 456–129 159) | 306 968 (199 825–471 512) | **.028** |
| Hb, g/dL | 8.2 (7.6–8.7) | 8.5 (4.6–12.4) | .703 |
| WBC, $\times 10^3$/µL | 9.8 (8.9–10.7) | 13.3 (6.4–20.3) | .110 |
| Glucose, mmol/L | 4.8 (4.2–5.3) | 4.8 (1.0–8.4) | .936 |
| Creatinine, µmol/L | 40.8 (24.6–57.0) | 27.8 (6.6–49.0) | .463 |
| Total bilirubin, mg/dL | 1.4 (1.1–1.8) | 0.9 (0.3–1.5) | .618 |
| Transfusion during acute malaria | 18 (27) | 2 (40) | .616 |

Data are mean (95% confidence interval) unless otherwise specified.

Abbreviations: GMPD, geometric mean parasite densities; IQR, interquartile range; WBC, white blood cells.

**Table 3. Markers of Bone Marrow Activity**

| | All | No Hemolysis on Day 14 | Hemolysis on Day 14 | *P* Value |
|---|---|---|---|---|
| Baseline RPI | 0.6 (0.5–0.7) | 0.7 (0.5–0.8) | 0.5 (0.3–0.8) | .632 |
| RPI Day 7 | 2.5 (2.1–2.9) | 2.7 (2.2–3.2) | 1.8 (1.3–2.4) | .529 |
| RPI Day 14 | 2.3 (2.0–2.7) | 2.4 (2.0–2.8) | 2.2 (1.0–4.3) | .989 |
| Baseline thrombocytes, $\times 10^3/\mu L$ | 102 (85–119) | 110 (92–130) | 105 (4–208) | .887 |
| Thrombocytes Day 7, $\times 10^3/\mu L$ | 396 (362–430) | 273 (139–407) | 403 (361–445) | .072 |
| Thrombocytes Day 14, $\times 10^3/\mu L$ | 340 (313–366) | 244 (81–407) | 346 (317–377) | .118 |

Data are mean (95% confidence interval).

Abbreviation: RPI, reticulocyte production index.

Erythropoietic activity in patients with and without delayed hemolysis was similar until the development of hemolysis on day 14 (Table 3). Furthermore, peripheral blood smear of patient H5 showed an adequate erythropoietic response for the severe anemia on day 14 with reticulocytosis and a large amount of normoblasts. There was no significant difference in platelet dynamics as a further marker of bone marrow activity.

In addition to the 5 children with delayed hemolysis, 3 children showed an increase in LDH (of 77 U/L, 140 U/L, and 236 U/L) to levels over 350 U/L (433 U/L, 857 U/L, and 413 U/L, respectively) without concurrent decline in hemoglobin.

Reasons for excluding 30 children from primary endpoint-analyses are shown in Supplementary Table 1. To reveal potential attrition bias due to selective lost to follow-up we compared baseline characteristics between patients included in the primary endpoint analysis and those not included. Patients not included were younger (median age: 33 months vs 39 months, $P = .005$) and had a higher initial parasitemia (GMPD: 226 296/μL vs 100 679/μL, $P = .008$) (Supplementary Table 2).

## DISCUSSION

This study provides evidence for delayed hemolysis in African children after treatment with parenteral artesunate for severe malaria. With an estimated frequency of around 7%, delayed hemolysis is a relatively frequent complication. The hemolytic activity determined by LDH was highest in those with the largest decline in Hb. Delayed hemolysis is also a highly clinically relevant complication as 1 patient had life-threatening hemolytic anemia on day 14, which required multiple blood transfusions and could not be attributed to another cause. Despite low hemoglobin levels, none of the other patients required subsequent blood transfusions beyond the initial hospitalization nor did they experience any obvious unfavorable outcome and would not have been detected passively as an adverse event without the implementation of standardized follow-up procedures.

Delayed hemolysis in this study is different from that seen during the acute phase of malaria or the hemolysis of blackwater fever (BWF), which remains an enigmatic clinical entity. In those cases where BWF is related to malaria treatment (mostly quinine), the time course between quinine use and hemolysis is much shorter. The median time between the beginning of treatment with quinine and BWF in a Vietnamese study was only 24 hours [21]. Delayed hemolysis, however, reaches its peak activity around day 14—with a rise in hemolytic activity already seen on day 7 [8–10]. Furthermore, BWF is mostly associated with absence of blood stage parasites or with very low parasitemia contrasting the observed association of the initial hyperparasitemia with delayed hemolysis described here [22]. Early-onset hemolysis has been described in association with parenteral artesunate, but it is difficult to separate hematologic effects associated with malaria from those associated with antiparasitic therapy during the acute phase [23].

To reliably distinguish hemolysis during the acute phase of malaria or BWF from delayed hemolysis, a complex definition of hemolysis requiring time-dependent laboratory-data on Days 7 and 14 was chosen. A strict definition is needed in a pilot study to increase the specificity of detecting delayed hemolysis. Using only LDH as a marker of hemolysis would considerably decrease specificity as it is an unspecific marker of cell destruction and inflammation. This is confirmed by three children in our study with a rise in LDH without concurring decline in hemoglobin. However, using such a strict definition leads to a lower sensitivity. Furthermore, because for some children no data or material was available for either day 7 or 14, a relatively high proportion of study participants were not included in the final analysis. Children excluded differed significantly from those included for some baseline characteristics. Therefore, an attrition bias cannot be excluded. However, the direction of such an attrition bias would lead to an underestimation rather than an overestimation of the frequency of the primary endpoint as excluded patients and those with delayed hemolysis shared the characteristics of being younger and having higher parasite densities.

The etiology of artesunate-associated delayed hemolysis remains unclear. A direct toxic effect of artesunate or its

**Table 4. Individual Characteristics of Patients With Signs of Hemolysis on Day 14**

| Patient | Gender | Age (Months) | Hb (g/dL) Day 0 | Hb (g/dL) Day 7 | Hb (g/dL) Day 14 | Hb (g/dL) Day 28 | Δ Hb (g/dL) (Day 14 - Day 7) | Δ LDH (U/L)) (Day 14 - Day 7) | Haptoglobin (mg/dL) | Parasitemia (/μL) | Site |
|---|---|---|---|---|---|---|---|---|---|---|---|
| H1 | F | 14 | 9.1 | 7.9 | 7.0 | 8.4 | −0.9 | 631 | 0.15 | 210 995 | Gabon |
| H2 | F | 24 | 4.1[a] | 6.8 | 6.0 | 10.1 | −0.8 | 460 | 0.15 | 391 465 | Ghana |
| H3 | M | 24 | 11.4 | 9.3 | 8.6 | 10.0 | −0.7 | 242 | 0.08 | 316 800 | Ghana |
| H4 | M | 63 | 11.3 | 9.5 | 9.0 | N/A | −0.5 | 118 | 0.04 | 223 125 | Ghana |
| H5 | M | 6 | 6.6[a] | 7.0 | 2.8 | N/A | −4.2 | 1013 | 0.15 | 466 745 | Ghana |

Abbreviation: Hb, hemoglobin.

[a] Patient received blood transfusion, post-transfusion Hb on same day: H2 6.8 g/dL; H5: 9.0 g/dL; Δ: change between day 7 and day 14.

metabolite dihydroartemisinin is unlikely due to half-lifes shorter than 2 hours [24]. Results presented here confirm previous findings obtained from nonimmune travelers with severe malaria in that mainly hyperparasitemic patients developed delayed hemolysis. This may indicate an association of delayed hemolysis with clearance and destruction of parasitized red blood cells. One possibility is that the time-delayed clearance of pitted erythrocytes may play a role, a splenic mechanism of removing parasitic structures out of infected red blood cells without destroying these [25]. These once-infected erythrocytes have a reduced life-span compared to naive erythrocytes—possibly resulting in a delayed peak of hemolysis after the initial destruction of infected (and noninfected) erythrocytes during acute malaria. Interestingly, the proportion of pitted erythrocytes is significantly higher after the use of artemisinins than after quinine, an effect that may explain that delayed hemolysis has not been observed with quinine [26, 27]. Another hypothesis is that immune-mediated hemolysis contributes to delayed hemolysis. Coombs testing in cases with delayed hemolysis in imported malaria published so far have remained inconclusive. In 3 patients in the case series by Zoller et al, Coombs tests were negative [10]. In our own report on 3 European travelers with delayed hemolysis after treatment with artesunate, 1 patient showed a positive Coombs test—albeit with an extremely low titer and with antibodies of anti-E specificity [9]. In the Belgian-Dutch cohort, 3 out of 6 of the tested patients had a positive Coombs test [8]. Delayed hemolytic transfusion reactions—in which transfused erythrocytes are destroyed after a latency of 2 days to 2 weeks due to alloimmunization during a prior transfusion—might have contributed to delayed hemolysis in 2 patients (Table 4). However, the extent of such a transfusion reaction is usually mild and without clinical implication and is unlikely to explain the dramatic fall in hemoglobin seen in patient H5 [28].

Finally, the fact that only a subset of patients developed delayed hemolysis may indicate that genetic susceptibility factors could play a role. One factor related to individual susceptibility could lie in different drug-metabolism patterns (eg, polymorphisms in CYP2A6)—but so far, few studies on artesunate and pharmacogenetics are available [29, 30]. Another factor of individual susceptibility could lie in red blood cell polymorphisms—such as sickle cell trait or G6PD deficiencies—which may predispose to hemolysis and are prevalent in malaria-endemic countries. As all affected children had a hemoglobin AA genotype, the influence of HbS or HbC seems unlikely. Furthermore, all boys with delayed hemolysis showed a wild-type G6PD genotype and the 2 girls a G6PD genotype correlating with normal or only mildly decreased enzyme activity, thereby rendering a contribution of G6PD deficiency to delayed hemolysis highly unlikely [20].

It is not possible to establish a causal relationship between artesunate and delayed hemolysis in the absence of a comparator group treated differently, for example, with quinine. Nevertheless, after years of extensive treatment with quinine, there are no reports on delayed hemolysis to our knowledge, and hemolysis seems to be restricted to patients treated with artemisinins [11]. With parenteral artesunate as the first-line antiparasitic therapy in severe malaria, it is difficult to establish a comparator group lacking suitable alternative antimalarials besides quinine.

As all children have additionally been treated with artemether/lumefantrine, one might argue that delayed hemolysis is associated with artemether/lumefantrine rather than artesunate or alternatively with the total dose of approximately 25 mg/kg of artemisinin drugs administered over only four to five days. In the past there have been some reports of delayed hemolysis after artemether/lumefantrine pointing to a possible class effect of artemisinins [31, 32]. As the pattern of hemolysis described in this study is following the same time course as that reported in European travelers who have not all been treated with an additional ACT, it is unlikely that artemether/lumefantrine is the sole cause of delayed hemolysis [8–10].

Artesunate has been shown to influence erythropoiesis with reports describing a decrease in the reticulocyte count for up to 2 weeks after application of artesunate in healthy volunteers [33]. Yet, we did not observe relevant bone-marrow suppression in patients with and without signs of hemolysis rendering

ineffective erythropoiesis unlikely as a major contributing factor to anemia in patients with delayed hemolysis in comparison to controls without hemolysis.

Compared to quinine, parenteral artesunate continues to be a relatively safe therapeutic option for this potentially lethal infection, despite the occurrence of delayed hemolysis. In view of its antiparasitic efficacy it is the drug of choice for severe malaria [5].

In conclusion, our findings demonstrate the existence of delayed hemolysis after parenteral artesunate for severe malaria in children in Africa. This may serve as a note of caution and prolonged follow-up within the first month after acute disease onset at least in patients with high parasite levels should be offered where possible while further pharmacovigilance is necessary. Results from this first prospective study should be used to plan future studies confirming the risk factors of delayed hemolysis and investigating its pathophysiologic basis.

## Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

## Notes

***Acknowledgments.*** The authors thank the participating children and their families, as well as the local investigators of the Severe Malaria in African Children (SMAC) network. The authors wish to thank the clinical laboratory of Dr Fenner and partners in Hamburg for supporting biochemical analyses.

Please see the local investigators listed in the appendix online.

***Contribution.*** T. R., B. M., P. G. K., S. K., G. D. B., and J. P. C. designed the study. T. A., S. I., A. A. A., P. G. K., and J. P. C. coordinated the study. T. A., S. I., J. S., D. A., D. S., A. A. A., S. Z. L., and P. G. K. represent the investigators of SMAC. J. M. performed genotyping. T. R. and J. P. C. analyzed results and wrote the first draft of the article, with all authors contributing subsequently.

***Financial support.*** This work was supported by the European & Developing Countries Clinical Trials Partnership (EDCTP; grant number: CT.2004.31070.001), the German Ministry for Education and Research (Bundesministerium für Bildung und Forschung, BMBF; grant number: 01KA1011), the Werner-Otto-Stiftung Hamburg (grant 7/81) and by a Clinical Leave Stipend to T. R. by BMBF via the German Centre for Infection Research (Deutsches Zentrum für Infektionsforschung, DZIF; grant 8000-202-2).

***Potential conflicts of interest.*** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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