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MAJOR ARTICLE

Post-artesunate Delayed Hemolysis in African Children With Severe Malaria: Incidence, Medical Impact and Prevention

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Background. Post-artesunate delayed hemolysis (PADH) occurs in 7%–25% of adults with severe imported malaria. Whether it exists in African children is controversial.

Methods. In total, 351 children treated with artesunate were enrolled in a prospective severe malaria study in Benin. Clinical, epidemiological and biological data, plasma concentrations of antimalarials were captured or determined on admission then at 3, 5, 14, 21, and 28 days after starting treatment. PADH was defined by a >10% drop in hemoglobin level and/or a >10% rise in LDH concentrations beyond Day 5.

Results. Fourteen children (4%) died before D14. Although 10% of guardians declared administration of anti-malarial drugs before admission, 316/350 (90%) of children had measurable plasma levels of lumefantrine (n = 279), quinine (n = 104), sulfadoxine (n = 67), artemisinin (n = 28), chloroquine (n = 16), or other antimalarials (n = 9). PADH occurred in 76/332 children (22.9%). Levels of pitted red blood cells (RBC) were higher and recovery from anemia was slower in these children. Severe anemia and transfusion were more frequent between D14 and D28 in children with PADH compared to children without PADH (10.6% vs 0.4%, 9.8% vs 0%). During follow-up, children with PADH were more frequently hospitalized (11.1% vs 1.6%) and had more frequent infectious events (6.9% vs 0.4%) than children without PADH. Children who received 2 transfusions within 3 days post-admission had a lower incidence of PADH than untransfused children (12.5% vs 26.8%, $P = .015$).

Conclusions. Despite widespread self-medication with antimalarials, PADH affects 23% of African children treated with artesunate for severe malaria, of whom more than 15% suffer from severe anemia and/or infectious events. Liberal early transfusion may be protective against PADH.

Keywords. severe malaria anemia; post-artesunate-delayed hemolysis; pitting; self-medication in African Children; PADH and transfusion.

BACKGROUND

Despite control efforts, malaria remains one of the main causes of child mortality and morbidity in sub-Saharan Africa [1]. Indeed, children younger than 5 were the victims of 76% of all malaria deaths in the African region in 2022 [1]. A significant part of these death is directly or indirectly associated with anemia [2]. Benin, like most countries in sub-Sahara, records malaria as the leading cause of morbidity and mortality with 4 938 668 cases and 11 154 deaths estimated in 2021 [3].

Intravenous (IV) artesunate is the World Health Organization (WHO)-recommended first-line option for severe *Plasmodium falciparum* malaria in adults and children [4], a life-saving treatment that is to be complemented by a full 3-day course of artemisinin-based combination therapy (ACT). Compared to quinine, parenteral artesunate reduces severe malaria-related mortality by 35% in adults and 22.5% in children [5]. Artesunate is safe and well tolerated. However, post-artesunate delayed hemolysis (PADH) is reported in 7%–25% of patients with severe malaria and also occurs (though rarely) in uncomplicated malaria treated

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with oral artemisinin derivatives [6–11]. These delayed episodes typically take place 5–21 days after starting therapy. This frequent adverse effect is well-documented in severe imported malaria in travelers. PADH is generally mild in adults although severe cases with complications have been reported with a proportion of affected patients requiring blood transfusion [6–8]. PADH has been associated with the clearance of "pitted" (once-infected) red blood cells (RBC) [6]. Once killed by artesunate, *P. falciparum* is expelled from its host RBC as it passes through the spleen by pitting [6]. Pitted-RBC then returns to the circulation. Due to their shorter life span compared to normal RBC, pitted-RBC are synchronously eliminated within a few days/weeks after treatment, triggering PADH in patients with high pitting rate [6]. In African children, artemisinin-induced parasite clearance is related or unrelated to pitting depending on the child's age and level of antimalarial immunity [12].

In children with severe malaria, few studies focused on PADH have been performed. In studies using different case definitions, authors reported an incidence of PADH ranging from 0% to 22% [9, 13, 14]. Because delayed hemolysis may negatively impact post-treatment recovery in this fragile pediatric population, a better knowledge of the incidence and impact of PADH is warranted [15]. Our objectives were to determine the incidence and impact of PADH in children with severe malaria in Benin.

METHODS

Study Design and Procedure

A prospective study was conducted from November 2020 to July 2021 in the pediatric department in the CHU-MEL hospital at Cotonou, Benin. This department manages severe malaria, severe pneumonia and severe wasting [16]. On arrival at the hospital with signs of severe malaria, a systematic malaria RDT was performed in a total of 1081 children aged ≤15 years. Positive RDTs were confirmed by flow-cytometry followed by a thick blood smear. In total, 351 children from different areas (Supplementary Fig. 1) infected with *P. falciparum* were enrolled after the consent of their parents or legal representatives. Children were not included in the study if they had hemoglobin ≤3 g/L, coma, severe active disease other than malaria, inflammatory bowel disease and history of chronic hemolytic anemia. A complete survey of medical history and previous medication was performed at admission (D0). Artesunate was administered based on body weight: 2.4 mg/kg for children weighing >20 kg and 3 mg/kg for those ≤20 kg, at admission (H0), 12 hours (H12), and 24 hours (H24), then once daily until the patient was able to take oral medication. Parenteral artesunate were completed by a full course of weight-adapted either with artemether-lumefantrine or with dihydroartemisinin-piperaquine. Plasma concentrations of antimalarials at D0, clinical and laboratory data and concentration of circulating pitted-RBC were captured or determined on admission then 3, 5, 14, 21, and 28 days after starting treatment. Quantitative and qualitative data were collected in KoboCollect™ software and Excel file.

Case Definition

Severe malaria was defined as an acute *P. falciparum* malaria with at least 1 sign of severity and/or evidence of vital organ dysfunction. The signs of severity were adapted from the WHO definition (Supplementary Table 1) [4]. Severe anemia was defined as a blood hemoglobin (Hb) level ≤5 g/dL in children <12 years and ≤7 g/dL in children ≥12 years. PADH was defined as a decrease in Hb (<10%) or an increase in lactate dehydrogenase (LDH) (>10%) after the first week and anytime during the follow-up until D28 [6].

Pitting Rate

Pitted-RBC cell was analyzed by flow cytometry (FACS CantoII™, BD Biosciences, USA). Briefly, erythrocytes were washed, fixed with 1% glutaraldehyde and permeabilized with 0.01% Triton X-100 (Sigma-Aldrich), and incubated with a nucleic acid dye (Hoechst 34580, Thermo Fisher™) and a human anti-RESA (ring-infected erythrocyte surface antigen) antibody, followed by an Alexa Fluor 488–conjugated goat anti-human immunoglobulin G (IgG) (Life Technologies, France) as previously described [12].

Drugs Screening

The plasmatic detection of drugs molecules at D0 were performed by the French Armed Forces Biomedical Research Institute. Analysis was carried out using an LC40 chromatograph (Shimadzu) coupled to a 4000 QTRAP mass spectrometer (Sciex). The sample preparation procedure consisted in a homemade protocol based on a solid phase extraction using 300 µL of plasma sample. The detected drugs associated to their limits of detection of this screening method are shown in Supplementary Table 2.

Hemoglobin Phenotyping

Identification of hemoglobin pattern was performed by the high-performance liquid chromatography (HPLC) variant NBS® (Bio-Rad) at Robert Debré University Hospital. Hemoglobin typing was assessed for samples at D3, or D0 when D3 was missing.

Statistical Analysis

Analyses were performed with R software version 4.0.3. Outliers and duplicates data were either corrected or recorded as missing. Descriptive characteristics were reported in terms of frequency for qualitative variables, mean or median, and standard deviation/minimum/maximum or interquartile range for quantitative variables. Analysis between the PADH/noPADH

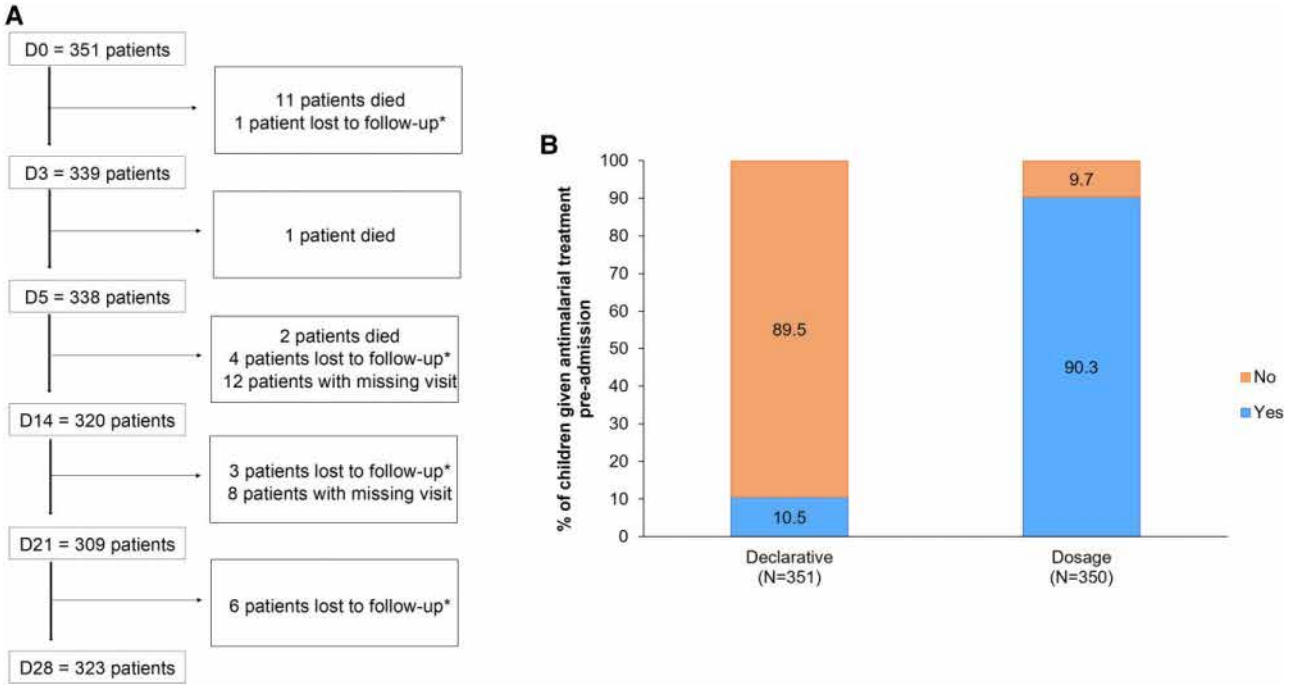

Figure 1. Flow Chart of the population and antimalarial treatment before admission. *A.* Follow up of the enrolled patients. *Children were considered lost to follow-up if they did not attend their last visit at day 28. *B.* Comparison between declarative and measure of antimalarial treatment. The presence of drugs before admission were recorded according to guardian declaration or determination of plasma levels on admission.

groups and the qualitative variables of interest were estimated by the χ^2 test or Fisher exact test if the test was non-parametric. The analysis between the PADH/noPADH groups and the quantitative variables of interest were estimated by Student's t test or Mann–Withney test if the test was non-parametric.

Ethical Considerations

The Nagoya procedure was submitted and was approved by the Beninese authorities (approval number 092/DFBCN/FP-APA/SA). The study protocol was approved by the Research Ethics Committee of the Institute of Applied Biomedical Sciences (CER-ISBA) (authorization number 132 of 12 August 2020). The parents/guardians of the children provided full, written, informed consent for participation. Protection and anonymity of patients was guaranteed by using identification numbers.

RESULTS

Characteristics of Enrolled Children

Of the 1081 patients screened, 351 children were enrolled in the study and 323 followed up to D28 (Figure 1*A*). A total of 14 children died during the study before D14. Of the 14 children who died, 9 (64.3%) had been referred from another health center, and 12 (85.7%) had been symptomatic for more than 72 hours. Compared to features in the whole cohort, the median age of these children was 36 vs 38 months, the median parasitemia was 150 380 p/μL vs 258 350 p/μL, and the median hemoglobin level at D0 was 54 vs 55 g/L. The main signs of severe malaria were impaired consciousness (81.5%), convulsions (49.9%), hyperparasitemia (39.9%) and severe anemia (21.9%) (Table 1). Given the high prevalence of sickle cell disease in Benin, we carried out a retrospective hemoglobin analysis in search of the sickle trait or sickle cell disease in these children. The hemoglobin profiles detected were: HbAA in 250/351 children (71.2%), HbAS in 54 children (15.4%), and HbAC in 33 children (9.4%). Some of the children were transfused before the D0 sampling and a mixed HbAS + HbAC detection occurred in 8 children (2.3%) and HbSS in 2 children (0.6%). The proportion of children with sickle trait or sickle cell disease was similar between children with PADH 20/75 (26.7%) and children without PADH 74/256 (28.9%) ($P = .7$).

Incidence of PADH and Impact of Early Transfusion

Of the 351 children enrolled, complete biological data was available in 332. The incidence of PADH was 22.9% (76/332 children). Parasitemia was higher in children who develop PADH (Supplementary Table 4). Most of the children (71.5%) received blood transfusion on arrival at the hospital due to anemia on admission (Table 1). Compared to children who were not transfused (n = 97), children who received 2 transfusions (n = 88) had a lower incidence of PADH (26.8% vs 12.5%, $P = .015$). A single transfusion had no protective impact (incidence of PADH = 26.5%, 39/147).

Table 1. Baseline Characteristics of Children on Admission (N = 351)

Variable	N (%)	Median (Q1-Q3)
Sex		
Female	162 (46.2)	…
Male	189 (53.8)	…
Age (y)		…
[0–2]	108 (30.8)	…
[2–5]	162 (46.1)	…
[5–15]	81 (23.1)	…
Body temperature (°C)		38.0 [37.5–38.8]
Transfusion on admission at CHU-MEL	251 (71.5)	…
Second transfusion on admission at CHU-MEL	96 (27.3)	…
Referral from another health center	190 (54.1%)	…
Signs of severe malaria		
Impaired consciousness	286 (81.5)	…
Convulsion	175 (49.9)	…
Hyperparasitemia (>10%)	140 (39.9)	…
Severe malarial anemia	77 (21.9)	…
Hypoglycemia	16 (4.6)	…
Pulmonary edema	1 (0.3)	…
Parasitemia (p/µL of blood)	…	258.350 [105.831–442.572]
Hemoglobin level (g/L) (N = 350)	…	55 [43–73]
Hematocrit level (%) (N = 350)	…	17.2 [13.1–22.7]
Lactate dehydrogenase level (U/L) (N = 341)	…	1.045 [802–1.564]
Platelets level (10^3/µL) (N = 350)	…	43 [27–70]
Mean corpuscular volume (fl) (N = 350)	…	80.2 [72.8–89.4]
Neutrophils level (N/µL) (N = 350)	…	6020 [3550–9040]
Reticulocytes (N/µL) (N = 340)	…	70.500 [47.000–106.250]

Table 2. Molecules Detected in the Blood of Children on Admission (N = 350)

Molecules	ACT[a]	N	%
Lumefantrine	Yes	279	79.71
Quinine	No	104	29.71
Sulfadoxine	Suspected	67	19.14
Dihydroartemisinin	Yes	23	6.57
Chloroquine	No	16	4.57
Pyrimethamine	Suspected	6	1.71
Artemether	Yes	5	1.43
Metabolic amodiaquine	Suspected	2	0.57
Piperaquine	Yes	1	0.29
Proguanil	No	0	0.00
Artemisinin	Yes	0	0.00
Mefloquine	Suspected	0	0.00

[a]Artemisinin-based combination therapy. The entry "Yes" corresponds either to artemisinin derivatives or molecules existing only in combination with an artemisinin derivative (ACT), the entry "No" corresponds to antimalarials that were not ACT, such as quinine and chloroquine and the entry "suspected" corresponds to the antimalarials that exist either alone or in fixed combinations with an artemisinin derivative. In such cases, we could not determine whether the child had been exposed or not to an ACT before admission.

Self-medication With Antimalarial Drugs

The mean concentration of pitted-RBC on admission for this cohort (2.5% of circulating RBC) is higher than previously reported in endemic or imported malaria. The presence of pitted-RBC generally indicates previous treatment with artemisinin derivatives before presentation [12, 17]. Although only 10.5% of guardians declared that their child had taken antimalarial drugs before admission, anti-malarial drugs were detected at D0 in 90.3% (316/350) of children (Figure 1*B*). Of these 316 children: 172 (54.4%) were referred from another health center immediately before their admission at CHUMEL, 37 (11.7%) reported having taken antimalarials, and 144 (41.1%) had 2–4 antimalarial drugs at detectable levels in their blood. We used the detection of antimalarials such as lumefantrine that are exclusively available in association with artemisinin derivatives as a surrogate for previous exposure to an ACT. Lumefantrine was found in 80% of children, followed by quinine in 30% and sulfadoxine in 19%. Other molecules found were dihydroartemisinin (DHA) (7%), chloroquine (5%), pyrimethamine (2%), artemether (1%), metabolic amodiaquine (0.6%), and piperaquine (0.3%) (Table 2). Compared to children who had no ACT-related drugs (DHA, artemether, lumefantrine, piperaquine) in their blood, children with detectable levels of such drugs had higher Hb at D0 (58 g/L vs 51 g/L, $P = .005$) and required less transfusion (68.3% vs 84%, $P = .025$) (Supplementary Table 3).

Evolution of Hematological Markers in Children With or Without PADH

On admission (D0), hematological parameters such as levels of hemoglobin, LDH, reticulocytes, and "pitted" RBC were similar regardless of whether children would later develop PADH (Figure 2 and Supplementary Table 4), with marked anemia in both groups (6 g/dL). After day 5 the kinetics of Hb and LDH followed the typical evolution of either PADH or the Rising pattern [6]. The hemoglobin level increased post-treatment and was significantly higher at Day 5 (80.7 g/L) in PADH group vs 74.8 g/L in group without PADH, $P < .05$). By contrast, the mean hemoglobin concentration decreased by 5.3% between D5 and D14 in the PADH group while it increased by 23.4% in children without PADH (Figure 2A1). The kinetics of LDH levels followed an inverted pattern that of hemoglobin ie, it was similar in both groups from D0 to D5, then increased only in the PADH group from D5 to D14 with a mean increase of 29.4% (Figure 2A2). The reticulocyte count was significantly higher in patients without PADH at D5 ($P < .05$) but then increased more significantly in children with PADH with a peak increase of 273% compared to 214% in children without PADH ($P < .0001$) (Figure 2A3). At D0, a high and unusual concentration of pitted RBC (2.5% of total circulation RBC) was observed in both groups of children. This pitting rate raised in both groups at D3 with a higher increase in the PADH group (10% of total RBC) compared to the children without PADH (7.2%, $P < .001$). This pitting rate remained significantly higher on D5 ($P < .05$) before drastically

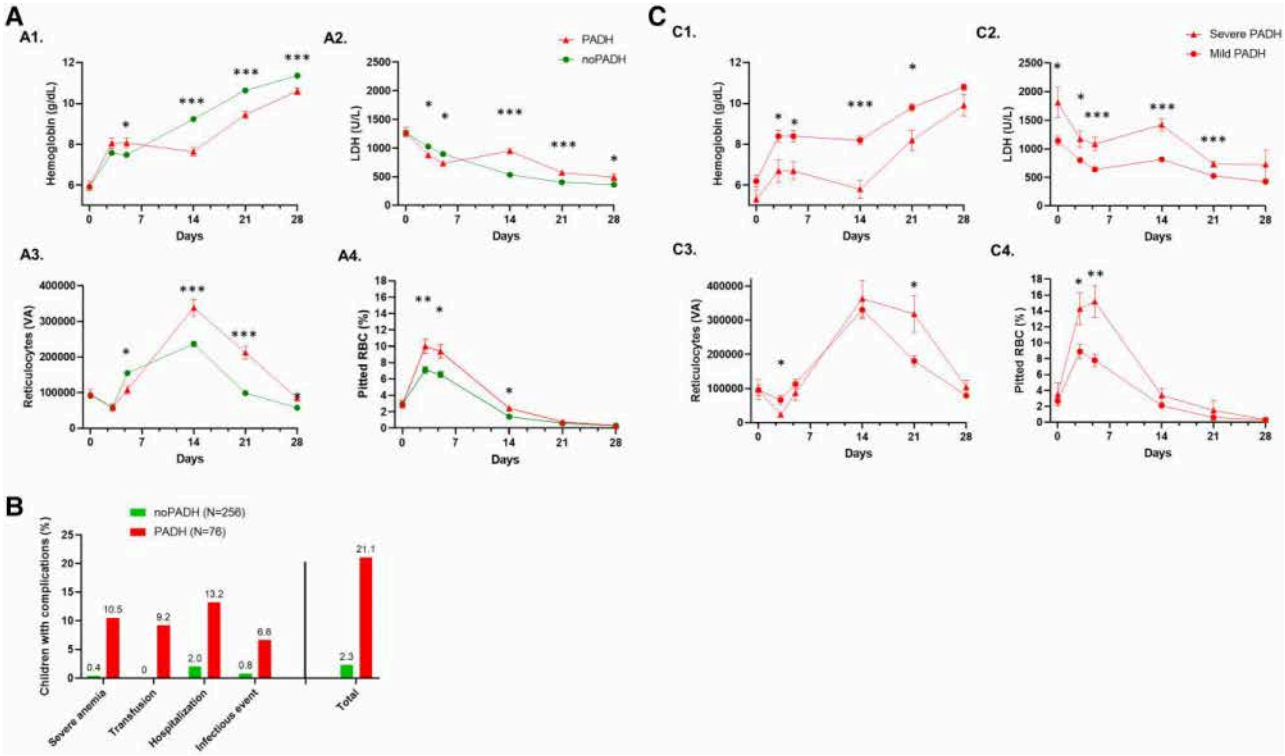

Figure 2. Hematological parameters and complications. *A.* Evolution of hematological parameters between children with (N = 76) or without (N = 256) PADH. Hemoglobin, LDH, reticulocytes count and pitted RBC were measured during the follow-up at day 0, 3, 5, 14, 21 and 28. Mean values (SEM), $*P < .05$, $**P < .001$, $***P < .0001$. *B.* Frequency of interventions or adverse events during follow-up after day 5 in children with or without PADH (N = 332). *C.* Evolution of hematological parameters between children with uncomplicated (mild), N = 60 vs complicated (severe) PADH, N = 16, i.e. with one or more complications such as infection, hospitalization, need for transfusion or severe anemia. Hemoglobin, LDH, reticulocytes count and pitted RBC were measured during the follow-up at day 0, 3, 5, 14, 21 and 28. Mean values (SEM), $*P < .05$, $**P < .001$, $***P < .0001$.

dropping from D5 to D14 in both groups (2.3% in PADH group and 2.1% in group without PADH) (Figure 2A4).

Medical Impact of PADH in African Children

To assess the potential impact of PADH, we compared the prevalence of signs, symptoms, intercurrent medical events, and interventions between D14 and D28 in children with or without PADH (Table 3). As expected, children with PADH were more frequently affected by severe anemia at D14 ($P = .003$) with over-prevalence of pallor at D21 ($P = .002$) and D28 ($P = .003$), deeper tachycardia at D14 ($P < .001$), more frequent intercostal indrawing at D14 ($P = .05$) greater need of blood transfusion at D14 ($P < .001$), and overall longer hospital stay ($P < .001$). Less expected was the higher incidence of infectious events (*Staphylococcus* or *Acinetobacter* infections, pneumonia, and sepsis) at D14 ($P = .003$). Concerning biological parameters, platelet level at D28 ($P = .035$), white blood cell level at D14 ($P < .001$) and neutrophil level at D14 ($P < .001$) were significantly higher in children with PADH compared with children without PADH. When complications and interventions such as severe anemia; transfusion; hospitalization; infectious events at D14, D21, and D28 were pooled their combined incidence was significantly higher in children with PADH than in those without PADH (21.1% vs 2.3%, $P < .0001$) (Figure 2*B*). These 21% of children with severe PADH displayed specific hematological parameters compared to the those with a mild PADH (Figure 2*C*).

DISCUSSION

Post-treatment complications contribute to the devastating impact of severe malaria worldwide and must therefore be adequately described to guide medical and public health decisions. We show here that, as previously observed in non-immune travelers, the incidence of post-artesunate delayed hemolysis (PADH) is high in African children, affecting more than 1 in 5. Unlike travelers, children with PADH are frequently affected by subsequent severe anemia or infectious events (or both) during follow-up, which may require specific post-treatment interventions to reduce these risks.

The high incidence of PADH in our study matches that suggested in a few but not all previous studies in African children. Delayed hemolysis following artemisinin-based therapy was

Table 3. Signs, Symptoms, Events and Interventions During Follow-up in Children With or Without PADH (N = 332)

	PADH (N = 76)[a]		No PADH (N = 256)[a]		
Variables	N (%)	Med (Q1-Q3)	N (%)	Med (Q1-Q3)	*P* Value
Severe anemia[b] at D14 (N = 320)	5 (6.9)	…	1 (0.4)	…	.003
Severe anemia[b] at D21 (N = 309)	1 (1.3)	…	0 (0.0)	…	.227
Severe anemia[b] at D28 (N = 322)*	2 (2.7)	…	0 (0.0)	…	.054
Pallor at D14 (N = 320)	62 (34.2)	…	213 (83.2)	…	.962
Pallor at D21 (N = 308)	35 (50.7)	…	73 (32.5)	…	.002
Pallor at D28 (N = 320)	16 (21.9)	…	22 (8.9)	…	.003
Tachycardia at D14 (N = 320)	6 (8.3)	…	0 (0.0)	…	<.001
Tachycardia at D21 (N = 308)	0 (0.0)	…	1 (0.4)	…	1
Tachycardia at D28 (N = 320)	0 (0.0)	…	0 (0.0)	…	/
Intercostal indrawing at D14 (N = 320)	2 (2.8)	…	0 (0.0)	…	.050
Intercostal indrawing at D21 (N = 308)	0 (0.0)	…	0 (0.0)	…	/
Intercostal indrawing at D28 (N = 320)	0 (0.0)	…	0 (0.0)	…	/
Transfusion at D14 (N = 320)	5 (6.9)	…	0 (0.0)	…	<.001
Transfusion at D21 (N = 309)*	2 (2.9)	…	0 (0.0)	…	.051
Transfusion at D28 (N = 323)	0 (0.0)	…	0 (0.0)	…	/
Hospitalization at D14 (N = 320)	8 (11.1)	…	4 (1.6)	…	<.001
Hospitalization at D21 (N = 309)	2 (2.9)	…	1 (0.4)	…	.130
Hospitalization at D28 (N = 323)	0 (0.0)	…	0 (0.0)	…	/
Infectious event at D14 (N = 320)	5 (6.9)	…	1 (0.4)	…	.003
Infectious event at D21 (N = 309)	0 (0.0)	…	1 (0.4)	…	1
Infectious event at D28 (N = 323)	0 (0.0)	…	0 (0.0)	…	/
Platelets at D14 (10^3/µL)	…	291 (214–417)	…	318 (246–403)	.247
Platelets at D21 (10^3/µL)	…	284 (197–367)	…	269 (211–346)	.442
Platelets at D28 (10^3/µL) (N = 322)	…	335 (260–431)	…	293 (228–376)	.035
WBC at D14 (10^1/µL) (N = 320)	…	913 (706–1 136)	…	636 (518–791)	<.001
WBC at D21 (10^1/µL)	…	755 (583–973)	…	685 (522–851)	.107
WBC at D28 (10^1/µL)	…	796 (634–1057)	…	745 (576–944)	.095
Neutrophils at D14 (10^1/µL) (N = 320)	…	248 (154–319)	…	182 (126–257)	<.001
Neutrophils at D21 (10^1/µL)	…	206 (137–321)	…	197 (139–279)	.547
Neutrophils at D28 (10^1/µL)	…	219 (143–340)	…	244 (177–314)	.322

[a]Values corresponding to the total number of patients analyzed: this number may vary and may be lower depending on the variables when there is missing data.

[b]Definition according to age.

**P* value at the 5% threshold limit. Univariate analysis.

described in 20–25% of adults with imported severe malaria [6–9, 12, 18, 19], with highest incidence (57.9%) in non-immune travelers of European origin [7]. Studies using variable case definitions in African children reported an incidence of post-treatment anemia ranging from 22% to 0% [9, 13, 20–22]. In a randomized controlled trial testing different regimens of artesunate, a post hoc analysis showed an incidence of post-treatment anemia of 22%, while a lower (5%) incidence of strictly defined PADH was reported in a randomized comparison of artesunate and quinine in hyperparasitemic children, possibly because these children had uncomplicated instead of severe malaria [14, 22]. In Uganda, Hawkes et al concluded on the absence of delayed hemolysis [20]. However, in this study, only 38% of the 91 children were followed until D14. In addition, 76% of children in this cohort were transfused < 30 minutes after their admission and there was no specific exploration in non-transfused children. By contrast, our study was specifically designed to capture all defining items of PADH and accounted for the early bone marrow response observed in African children [2].

PADH affects travelers and African children with similar incidence, but in markedly different pathophysiological and therapeutic contexts. Severe anemia is rare in adults with imported severe malaria—anemia is not even constant on admission-, and reticulocyte counts peak at D21 [6, 23]. By contrast, in children with malaria, profound anemia is the most frequent severity-defining complication, and reticulocyte response takes only a few days to emerge. Also, splenomegaly is rare in travelers, whereas it is frequent and closely correlated to severe anemia in African children [16, 24]. RBC loss due to spleen congestion has been recently shown to be a major determinant of malarial anemia in endemic areas [25]. Malaria-related anemia with spleen congestion leads to a self-amplifying anemia-splenomegaly-anemia loop where splenic retention of stiff RBC is faster when splenomegaly is present [26]. In African children with severe anemia, the "hungry" spleen likely

contributes to PADH and "post-PADH" events revealed by our study. In addition to anemia, spleen congestion may indeed also induce a relative hyposplenism [27] that hampers an adequate response to infection, explaining the a subgroup of severe PADH with higher incidence of sepsis, pneumonia and documented infections (6.6%). Free iron released by hemolysis may have also induce tissue-specific oxidative damage and favored infection [28]. Exposure to antimalarials before admission may also impact post-treatment evolution. We observed a striking contrast between the 10% of guardians who declared preadmission therapy and the 90% of plasma samples containing antimalarials. Malaria was therefore almost constantly self-treated before admission in this study, often by artemisinin-containing regimens. This explains the high prevalence of children carrying pitted RBC on admission [12, 17], a very rare observation in travelers [29]. This high rate of pitted RBC may however contribute to the magnitude of RBC lost during PADH. Of note, widespread self-treatment with artemisinin-containing antimalarial regimens likely contributes to the semi-recent emergence of parasite resistance to artemisinin in Africa.

More than two thirds of children received blood transfusion due to anemia on admission, and when a liberal transfusion policy was adopted, it was associated with a >50% decrease in the incidence of PADH. Further studies are needed to validate if the immediate lifesaving effect of transfusion in severe malarial anemia [2] may be complemented by the second significant benefit of preventing PADH. The potential mechanism by which transfusion of RBC reduces the risk of PADH is intuitive, that is, the dilution of pitted and altered RBC that will be cleared during the delayed hemolytic episode. Why only the liberal "double transfusion" was protective and not the single transfusion regimen is more difficult to explain. We suspect that the anemia-splenomegaly-anemia self-amplifying loop may be triggered only when a given threshold of splenic congestion is reached, jamming of the filtering beds becoming tight enough to induce the retention of normal RBC. That congestion of malarial spleens is made very predominantly of uninfected RBC [15, 20, 24], and microfluidic observations of normal RBC being trap in slits occupied by parasitized RBC strongly support this hypothesis [30]. Comparative studies more robustly designed than our observational approach will confirm or infirm this transfusion-preventive effect, its dose-dependence and, hopefully, will determine the most resource-effective regimen. If confirmed, this observation will further reinforce the importance for malaria-endemic countries to support functional and safety blood banks. Despite its open, non-comparative design, and the posthoc integration of drug dosing data, and lack of spleen imaging explorations, our study closes the controversy regarding the existence of PADH in African children treated with artesunate for severe malaria and opens the way for a robust preventive approach, liberal transfusion on admission. Planning surveillance at D14 could also be very useful to avoid serious complications not being identified and treated.

Supplementary Data

Supplementary materials are available at *Clinical 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.

Notes

Author contributions. P. A. N. designed, conceptualized, acquired funding, supervised and coordinated the overall project as principal investigator, analyzed, interpreted and validated the data, wrote, edited and reviewed the manuscript. V. C. coordinated the study and monitored databases, collected and analyzed data, wrote and reviewed the manuscript. N. F. designed and coordinated the field study, administrated, and performed experiments and reviewed the manuscript. J. Y. S., J. A., N. F., N. T., and P. B. designed the study, analyzed and interpreted the data, and reviewed the manuscript. P. B. wrote and edited the manuscript. D. S. performed experiments, analyzed data, wrote and reviewed the manuscript. C. C. performed experiments, trained people in Benin and in Paris, and reviewed the manuscript. C. L. and N. T. performed the drugs screening in plasma. C. L., A. F., O. N., E. A., R. A., A. B. D., B. A., and N. T. performed experiments, reviewed the manuscript. A. Y. coordinated the whole hospital study, collected, analyzed, interpreted data, and reviewed the manuscript. F. G., N. C., and R. O. performed statistical analysis and reviewed the manuscript. A. M., B. K., and A. T. provided technical support, analyzed and reviewed the manuscript. E. M. B. and A. Y. collected data. P. H. monitored databases and coded the CRF on Kobocollect software. V. C. and D. S. contributed equally. N. F. and A. Y. contributed equally. J. Y. S., N. D., P. B. and J. A. contributed equally.

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Data availability. Due to confidentiality agreements with research collaborators, supporting data (deidentified participant data, data dictionary, study protocol [in French]) can only be made available to bona fide researchers subject to a non-disclosure agreement. Data can be requested on demand to the corresponding author using email addresses, and data will be shared after approval by the 4 members of the OPTIMA consortium.

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