Post-malarial anemia in Mozambican children treated with quinine or artesunate: A retrospective observational study

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ABSTRACT

Objectives: This retrospective analysis performed in Manhiça, Southern Mozambique, aimed to describe the frequency of post-malarial anemia (measured as a decrease of hematocrit $\geq$10%) and the need for blood transfusions in children with severe malaria treated with intravenous quinine or parenteral artesunate.

Methods: All children <15 years admitted with a parasitologically-confirmed diagnosis of malaria from 1st January 2003 to 31st December 2017, alive at hospital discharge, and with at least one measurement of hematocrit within 28 days after hospital discharge, detected by passive case detection, were included.

Results: The overall prevalence of post-malarial anemia observed in the study was 23.13%, with an estimated incidence rate of 288.84 episodes/1,000 children-month at risk in the follow-up period (28 days after discharge). There were no differences between treatment groups, although the study showed a higher association between blood transfusions and artesunate treatment.

Conclusions: In this setting, children with severe malaria frequently present a meaningful decrease of hematocrit (>=10%) in the first weeks after their episode, sometimes requiring blood transfusions. Because of the high underlying prevalence of anemia in malaria-endemic settings, all children with severe malaria need to be actively followed up, irrespective of the treatment received.

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Introduction

Malaria remains one of the most important global parasitic diseases, causing 219 million cases and around 435 000 deaths in 2017 (WHO, 2019). This overwhelming burden makes the optimal management of malaria a global health priority. In terms of curative efficacy, parenteral artesunate is undoubtedly superior to quinine (Dondorp et al., 2005, 2010), and thus is currently recommended globally as the standard of care for the treatment of severe malaria. Meta-analyses have also confirmed that artesunate has a better short-term safety profile than quinine (Sinclair et al., 2012). However, there is a paucity of data on the

mid-term adverse events after the treatment period. Furthermore, in real-life conditions, late complications after discharge may be undetected under routine health assistance, considering the fragile health infrastructure in most countries where malaria is highly endemic (Rolling et al., 2014). Consequently, it was not until the introduction of parenteral artesunate as the first-line treatment for imported malaria in non-endemic countries that reports of post–artesunate delayed hemolysis (PADH) emerged (Rolling et al., 2015). These reports have shown clinically relevant PADH occurring in around 15-30% of patients, typically peaking 2-4 weeks after treatment, causing a frequent need for blood transfusions in the most severely ill patients (Gomez-Junyent et al., 2017; Jaureguiberry et al., 2015; Kreeftmeijer-Vegter et al., 2012; Kurth et al., 2017; Lahoud et al., 2015; Rehman et al., 2014; Rolling et al., 2015; Rolling et al., 2013; Roussel et al., 2017; Zoller et al., 2011). The primary mechanism behind these episodes of hemolytic anemia probably relates to the splenic clearance of erythrocytes by pitting (Arguin, 2014; Jaureguiberry et al., 2014). However, evidence generated from non-endemic countries cannot be directly generalized to endemic countries where vulnerable age groups, patient characteristics, clinical manifestations, or quality of care, among other things, may significantly differ (Cramer et al., 2011).

Notably, among specific populations of endemic countries, such as for instance young children, anemic episodes related to malaria treatment could have a profound and synergistic impact, due to the underlying concomitant conditions that are already highly prevalent in these settings, and that are known contributors to the high prevalence of anemia, including iron deficiency, hemoglobinopathies, malnutrition or chronic infections (Kassebaum et al., 2014; Moraleda et al., 2017). Data from different malaria studies conducted so far among African children show a lower incidence of post-malarial anemic events than in patients from non-endemic areas (Burri et al., 2014; Fanello et al., 2017; Hawkes et al., 2019; Rolling et al., 2014; Sagara et al., 2014; Scheu et al., 2019). However, the scarce data available regarding the mid-term safety in endemic areas of a drug so widely utilized is of concern, as such a potential side-effect could have a significant public-health impact in countries where malaria and anemia co-exist, mainly because safe blood products are not readily accessible.

This retrospective analysis aimed to determine, in a rural setting in Mozambique, whether the use of parenteral artesunate in comparison with intravenous quinine, for the treatment of malaria in children, was associated with a higher occurrence of post-malarial anemia (defined as a decrease of hematocrit values >=10%) and a higher need for blood transfusions.

Methods

Study design

This is a retrospective analysis of data collected through the Manhiça District Hospital (MDH) outpatient and inpatient pediatric morbidity surveillance system (MSS). All children under 15 years admitted with a diagnosis of malaria from 1 st January 2003 to 31st December 2017, confirmed to be discharged alive from the hospital, and with at least one measurement of hematocrit within 28 days after hospital discharge detected by passive case detection, were included.

Study setting

Mozambique is one of the ten countries with the highest malaria endemicity in the world, accounting for about 4% of the total global malaria prevalence (WHO, 2019). Mozambique's entire population, estimated to be around 29 million people, is at risk of malaria, and estimates suggest that in 2017, there were approximately 10 million cases and 14,700 estimated deaths (WHO, 2019). In-country parasite prevalence rates are variable but can range from <3% to more than 50%. Artesunate has been recommended as the first-line drug for severe malaria in Mozambique since 2011 (although the drug did not become fully available until 2013), and the shift from quinine to artesunate has gradually occurred across the country (Armindo Daniel Tiago et al., 2011).

This study was conducted in Manhiça, Southern Mozambique. For the past 20 years, the *Centro de Investigação em Saúde de Manhiça* (CISM; Manhiça Health Research Centre) has been running a demographic surveillance system (DSS), and around-the-clock MSS at the neighboring MDH, which sees around 75,000 pediatric outpatients and admits an average of ≈S3,000 children annually (Sacoor et al., 2013). Malaria in Manhiça district is perennial, although with a clear seasonality (November-April), coinciding with the rainy season. The district's malaria incidence has markedly changed in the last two decades, ranging from a high initial transmission period (2003–2007) to a moderate-to-low one after that. The leading causes of admission and under-five mortality in Manhiça are malaria, pneumonia, diarrhea, malnutrition, and neonatal pathologies (Sacarlal et al., 2009). The prevalence of anemia in children admitted to MDH has been proven to be high, with undernutrition, iron deficiency, HIV infection, and malaria being the main contributors identified (Moraleda et al., 2017). Data from the Manhiça district, generated by CISM using the outpatient and inpatient pediatric MSS databases, confirm different micro-epidemiological and clinical malaria patterns within its study area (Bassat et al., 2008; Guinovart et al., 2008). A comprehensive characterization of MDH, CISM, and the study area can be found elsewhere (Sacoor et al., 2013).

Hospital surveillance system

Standardized outpatient and admission questionnaires, which include demographic, clinical, laboratory, and outcome data, are routinely completed for all children <15 years of age attending or being admitted to MDH. On arrival, all children with documented fever (>=37.5 °C, axillary), a history of fever in the preceding 24 hours, or suspected anemia, are given a finger-prick blood sample to measure packed cell volume (PCV); thick and thin blood films are prepared or histidine-rich protein 2 (HRP2)-based rapid diagnostic tests (RDT) are conducted to screen for *P. falciparum* infection. HIV status information is not routinely collected. Once the child is discharged alive or has a fatal outcome, up to four final diagnoses, based on the International Classification of Diseases system version 10 (ICD-10), and treatments received are recorded in the questionnaire after reviewing all available results.

Laboratory methods

PCV was measured using a microcentrifuge and a Hawksley hematocrit reader card (Hawksley and Sons Ltd., Lancing, United Kingdom). Thick and thin blood films for malaria diagnosis were processed as previously detailed (Bassat et al., 2008; Bassat et al., 2009). The Lambaréné method, which counts parasites against an assumed known blood volume, is the method used to calculate parasitemia (Planche et al., 2001), which is considered negative if no parasites are detected after examination of 200 oil-immersion fields in a thick blood film. For routine clinical management, CISM's laboratory uses a semiquantitative "cross' system, ranging from 0 (no malaria infection) to 5 (high parasitemia infection) (WHO, 1999).

Definitions

All case definitions were based on admission data from the standardized questionnaires. A malaria case was defined as a child admitted with fever or a history of fever in the preceding 24 hours, *P. falciparum* asexual parasitemia > 0 parasites/μL (1- 5 crosses) (WHO, 1999), and a clinical diagnosis of malaria provided by the discharging clinician. Severe malaria cases were defined according to World Health Organization (WHO) definitions, as previously described (Bassat et al., 2008). The Blantyre coma score (BCS) was used to characterize consciousness. Deep coma and impaired consciousness were defined for BCS ≤ 2 and BCS < 5, respectively. Repeated convulsions were defined when occurring two or more times in a day. Prostration was defined as the inability to sit unaided or look for a mother's breast/feed in children who were not yet able to sit. Respiratory distress was defined as deep breathing or indrawing. Hypoglycemia was defined as glycemia <2.2 μmol/L (WHO, 2014). Moderate anemia was defined as hematocrit < 42% for children ≤28 days and hematocrit < 33% for children > 28 days. Severe anemia was defined as hematocrit < 25% for children ≤ 28 days and hematocrit < 15% for children > 28 days. Post-malarial anemia was defined as a decrease of at least 10% from the hematocrit value at the initial hospital admission for any hematocrit value determined within 28 days after discharge. Rather than considering the initiation of treatment as the start of follow-up, as proposed by Jauréguiberry et al. (Jaureguiberry et al., 2014), we have chosen discharge as the beginning of follow-up for two main reasons: (1) A lack of hematocrit data from admitted patients beyond recruitment because these are not routinely collected and not included in the standardized admission questionnaires; and (2) The short mean length of admission (quinine group: 3.05 days (95% CI: 2.94 -3.16); and artesunate group: 2.62 days (95% CI: 2.14-3.10) (p-value: 0.0173)) with very few episodes leading to hospitalizations longer than seven days. As we considered this decrease as a non-recurring event, the analysis was restricted to the first episode of a decrease of hematocrit ≥10% in children treated for severe malaria (Arguin, 2014). In the absence of systematic measurements for every child, laboratory markers of hemolysis as lactate dehydrogenase or haptoglobin values (not routinely available in this rural setting), or etiological data of anemia such as iron deficiency, hemoglobinopathies, bacteremia, viral infections (Parvovirus B19, Ebstein Barr Virus, HIV) or intestinal parasitic infections, it was only feasible to describe the general occurrence of anemia (irrespective of type) after treatment in this population. Nutritional status was assessed using anthropometrical Z-scores.

Case management

Children diagnosed with malaria were managed according to Mozambican national guidelines. During the initial study period (January 2003–September 2006), first-line treatment for uncomplicated malaria included amodiaquine plus sulfadoxine-pyrimethamine (SP). In September 2006, this changed to artesunate plus SP and from 2009 onwards to artemether-lumefantrine, Coartem®). From January 2003 to May 2013, the first-line treatment for severe malaria included parenteral quinine (with an initial loading dose of 20 mg/kg plus subsequent 10 mg/kg doses, three times a day) for a minimum of six doses if completed with treatment with SP, or 21 doses when used as monotherapy. Treatment was switched to oral as soon as the child clinically improved and was able to tolerate it. In this period, artesunate was not available in Manhiça. In 2013, quinine was progressively replaced by artesunate (2.4 mg/kg immediately, then at 12, 24 h and then once daily until oral medication could be taken reliably, for a minimum of three doses). In 2015, and for children weighing

less than 20 kg, the dose was increased to 3 mg/kg following an update in WHO recommendations (WHO, 2015). Blood transfusions were restricted to children with a PCV < 12%, hemoglobin <4 g/dL (when available), or to children with higher values but with clinical signs of decompensation (respiratory distress or signs of heart failure) or neurological impairment (Bassat et al., 2008). Facilities for intensive care are not available at MDH. All clinical assistance and treatment of admitted children are free of charge. Children requiring specialized care were transferred to Maputo Central Hospital.

Data management and statistical methods

This study includes all children under 15 years admitted with a diagnosis of malaria during 2003-2017, alive at hospital discharge, and with at least one measurement of hematocrit within 28 days from hospital discharge, detected by passive case detection.

Qualitative variables were compared using a χ^2 test or Fisher's exact test. Quantitative variables were compared using the Student t-test. Variables with statistically significant differences between treatment groups were used for model estimates adjusted for imbalances in baseline characteristics in children with post-malarial anemia.

Incidence rates were reported as the number of events per 1000 Children-month at risk (CMAR), with 95% confidence intervals (CIs). Differences in time to first-or-only episodes within 28 days after hospital discharge between treatment groups were analyzed by the Log-rank test and Cox regression. Negative binomial regression models were estimated to compare incidence rates of multiple episodes between treatment groups. The association of treatment with at least one episode within 28 days after hospital discharge was assessed by logistic regression models. Also, the equivalence of the cumulative incidence functions between the two treatment groups, accounting for the risk of dying before reaching specified outcomes, was assessed by the weighted log-rank test as previously proposed (Fine and Gray, 1999); competing risk regression of sub-hazard ratios was done according to Gray (Gray, 1988) with death as the competing risk to evaluate the association between outcomes and treatment adjusted for other covariates.

Anthropometrical Z-scores were computed using the LMS method and the British and WHO Child Growth Standards composite data file for term births implemented by the zanthro command in Stata (StataCorp, 2017). Statistical comparisons were performed at a two-sided significance level of 0.05, and 95%. Confidence Intervals were calculated for all estimations. All analyses were performed using Stata/SE software version 14.1 (StataCorp, 2017)

Ethical approval

This study retrospectively assessed data collected in the context of routine clinical practice. The MSS in place at MDH has been approved by the National Bioethics Committee for Health of Mozambican (CNBS-IRB00002657). The analytical plan of this specific analysis was assessed and approved by CISM's Internal Scientific Committee.

Results

During the 15-year study period (1st January 2003 to 31st December 2017), 23523 children <15 years of age were admitted to MDH. On admission, 9523/23523 (40.48%) children had a malaria diagnosis according to the case definition. Among these, 9461/9523 (99.34%) were alive at discharge; 62/9523 (0.65%) hospital deaths were registered. Fifty-five out of the 62 malaria deaths were
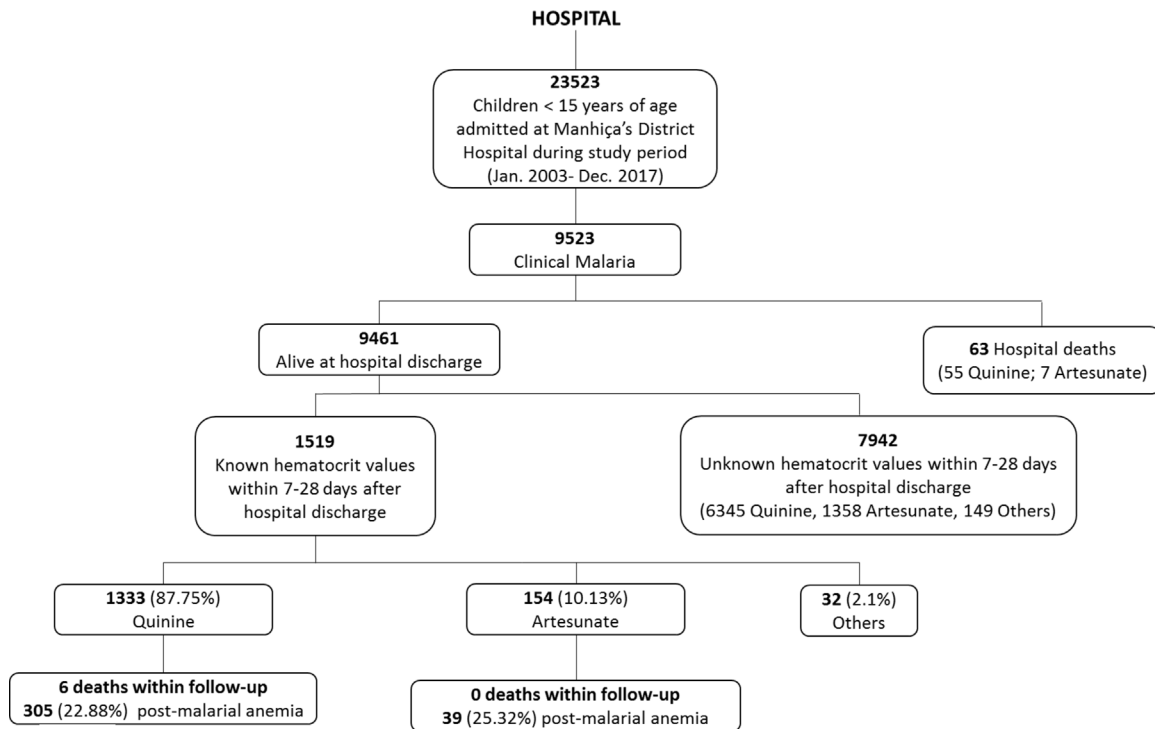
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Figure 1. Study profile.

children treated with quinine, and five out of 62 were treated with artesunate (Figure 1). Of those alive, 1519 (16.05%) children had at least one passive case detection contact with the MSS, including at least one hematocrit value detected within the 28 days after hospital discharge. There were 7942 children with unknown values of hematocrit (6345 quinine, 1358 artesunate, 149 others). Children without hematocrit data were excluded from the analysis. Of those with hematocrit data, 1333 (87.75%) had initially received quinine for their malaria treatment, 154 (10.13%) parenteral artesunate, and 32 (2.1%) other treatments.

Baseline characteristics of children by treatment group (univariate analysis)

Table 1 compares the baseline characteristics of admitted children treated with artesunate or quinine. Values of hematocrit during their original admission and proportion of malaria cases with severe anemia were similar in both groups. Children treated with quinine tended to have higher respiratory and heart rates (p < 0.0001, both); and they also had a higher percentage of palpable spleens (p < 0.0001). Children receiving artesunate were more prone to present with impaired consciousness and deep coma (p = 0.0115 and p = 0.0190).

Prevalence of post-malarial anemia

Of those children with known hematocrit values within 28 days after hospital discharge, 154/1519 (10.13%) had been treated with artesunate, and 1333/1519 (87.75%) had received quinine (Figure 1). No differences were observed in terms of the prevalence of post-malarial anemia according to the treatment group: 22.8% (305/1333) in the quinine group vs. 25.32% (39/154) in the artesunate group (OR = 1.14, 95% CI = 0.78, 1.68; p-value: 0.4962) (Table 2).

Post-malarial anemia incidence rates

The overall incidence rate of post-malarial anemia episodes throughout the study period was 288.84 episodes/1000 CMAR (CI: 259.88, 321.04). There were no differences in the incidence of anemia between children receiving artesunate and those treated with quinine (285.42 episodes/1000 CMAR vs. 318.8 episodes/1000 CMAR; Hazard ratio (HR): 1.12, 95% CI: 0.81-1.57; p-value: 0.4879) (Table 3). Mean time to post-malarial anemia episode was 16.89 days (95%CI: 16.06-17.72) in the artesunate group and 16.74 days (95%CI: 14.40-19.08) in the quinine group, without statistical difference (p-value: 0.9078). The cumulative incidence curve estimates for time to the first-or-only episode of post-malarial anemia did not show any differences according to treatment received (Weighted log-rank test for the Cumulative Incidence of anemia episode: Chi2(1) = 0.48; p-value = 0.4877) (Figure 2).

Given that this was not a randomized control trial, it would appear essential to adjust the comparison to potential existing confounders. The adjusted analysis further explored the differences between the two treatment groups, identifying splenomegaly as the single factor that remained independently associated with post-malarial anemia during the 28 period days post-discharge (Supplementary material, tables 1 and 2), with no evidence of other significant differences.

Blood transfusions

The overall rate of blood transfusions during the follow-up period was 20.64 episodes/1,000 CMAR (CI: 14.15, 30.09). Artesunate-recipients showed a higher rate when compared to the quinine group (6/154 vs. 21/1133; 44.73 episodes/1,000 CMAR vs. 17.88 episodes/1,000 CMAR, respectively) (HR: 2.50; CI: 1.01–6.19; p-value: 0.0479) (Table 4). This difference was still statistically significant when adjusting for other variables (supplementary material, Table 3).

Table 1
Univariate analysis of clinical variables and diagnosis according to treatment group.

Variable		Treatment		Total (N = 1487)	p-value
		Quinine (N = 1333)	Artesunate (N = 154)		
Sex [1]	Male	701 (53%)	74 (48%)	775 (52%)	0.4438[2]
	Female	631 (47%)	76 (49%)	707 (48%)	
Age at discharge [1]	0-<1y	290 (22%)	15 (10%)	305 (21%)	< 0.0001 [2]
	1y-<5y	922 (69%)	100 (65%)	1022 (69%)	
	5y<-15y	121 (9%)	39 (25%)	160 (11%)	
Parasitemia at admission [1]	Low (1-2 crosses)	126 (9%)	10 (6%)	136 (9%)	< 0.0001 [2]
	Medium (3-4 crosses)	650 (49%)	43 (28%)	693 (47%)	
	High (>4 crosses)	557 (42%)	101 (66%)	658 (44%)	
Length of admission (days) [3]		3.05 (2.94, 3.16) [1333]	2.62 (2.14, 3.10) [154]	3.00; (2.90, 3.11) [1487]	0.0173 [4]
Weight (kg) [3]		11.34 (11.08, 11.61) [1332]	13.83 (13.04,14.63) [154]	11.60; (11.34,11.85) [1486]	< 0.0001 [4]
Height (cm) [3]		83.80 (82.89, 84.72) [1217]	92.63 (89.61, 95.65) [135]	84.69; (83.80, 85.57) [1352]	< 0.0001 [4]
BMI: Body Mass Index (kg/m^2) [3]		15.83 (15.54, 16.13) [1216]	15.64 (15.30, 15.98) [135]	15.82; (15.55, 16.08) [1351]	0.6673 [4]
Weight for age z-score [3]		−0.90 (−0.97, -0.84) [1312]	−0.91 (-1.10, -0.72) [149]	−0.90; (−0.97, -0.84) [1461]	0.9587 [4]
Length/height for age z-score [3]		−0.96 (−1.05, -0.87) [1185]	−0.97 (-1.23, -0.71) [129]	−0.96; (−1.05, -0.88) [1314]	0.9673 [4]
BMI for age z-score [3]		−0.36 (−0.44, -0.28) [1182]	−0.27 (-0.51, -0.03) [130]	−0.35; (−0.42, -0.27) [1312]	0.4979 [4]
Malnutrition as a secondary diagnosis (E40-E46 at ICD-10) [5]		34 / 1333 [3%; (2, 4)]	0 / 154 [0%; (0, 2)]	34 / 1487 [2%; (2, 3)]	0.0450 [2]
Palpable spleen [5]		355 / 1332 [27%; (24, 29)]	17 / 153 [11%; (7, 17)]	372 / 1485 [25%; (23, 27)]	< 0.0001 [2]
Palpable liver [5]		17 / 1332 [1%; (1, 2)]	3 / 153 [2%; (0, 6)]	20 / 1485 [1%; (1, 2)]	0.4523 [6]
Temperature [3]		38.37 (38.29, 38.44) [1328]	38.57 (38.37, 38.77) [153]	38.39; (38.32, 38.46) [1481]	0.0727 [4]
Heart rate [3]		132.75 (131.34, 134.16) [1313]	113.37 (109.58, 117.16) [154]	130.71; (129.36, 132.07) [1467]	< 0.0001 [4]
Respiratory rate [3]		41.06 (40.46, 41.66) [1325]	35.88 (34.29, 37.46) [154]	40.52; (39.95, 41.09) [1479]	< 0.0001 [4]
Glycemia (mmol/L) [3]		6.12 (5.95, 6.29) [1257]	6.29 (5.98, 6.60) [118]	6.13; (5.98, 6.29) [1375]	0.5549 [4]
Hematocrit at admission [3]		27.29 (26.91, 27.68) [1333]	27.98 (26.83, 29.14) [154]	27.36; (27.00, 27.73) [1487]	0.2581 [4]
Severe anemia (PCV < 25% if < 28 days, PCV < 15% if ≥ 28 days) [5]		34 / 1333 [3%; (2, 4)]	5 / 154 [3%; (1, 7)]	39 / 1487 [3%; (2, 4)]	0.5916 [6]
Deep Coma (BCS ≤ 2) [5]		11 / 1332 [1%; (0, 1)]	5 / 154 [3%; (1, 7)]	16 / 1486 [1%; (1, 2)]	0.0190 [6]
Impaired consciousness (BCS < 5) [5]		42 / 1332 [3%; (2, 4)]	11 / 154 [7%; (4, 12)]	53 / 1486 [4%; (3, 5)]	0.0115 [2]
Repeated convulsions (≥2/24 h) [5]		73 / 218 [33%; (27, 40)]	7 / 35 [20%; (8, 37)]	80 / 253 [32%; (26, 38)]	0.1112 [2]
Hypoglycemia (<2.2 mmol/L) [5]		16 / 1260 [1%; (1, 2)]	1 / 120 [1%; (0, 5)]	17 / 1380 [1%; (1, 2)]	1.0000 [6]
Prostration [5]		150 / 1333 [11%; (10, 13)]	18 / 154 [12%; (7, 18)]	168 / 1487 [11%; (10, 13)]	0.8716 [2]
Respiratory distress [5]		98 / 1332 [7%; (6, 9)]	6 / 154 [4%; (1, 8)]	104 / 1486 [7%; (6, 8)]	0.1109 [2]

1: n (Column percentage). 2: Chi-squared test. 3: Arithmetic Mean (95% Confidence Interval) [n]. 4: t-test. 5: n [Column percentage; (95% Confidence Interval)]. 6: Fisher's exact test

Table 2
Prevalence of anemia within 28 days after discharge.

Treatment	Clinical malaria admissions	Anemia episodes	Rate estimations		Model estimations		
			Prevalence (%)	95% ConfidenceInterval	Odds Ratio	95% Confidence Interval	p-value
Quinine	1333	305	22.88	(20.65, 25.23)	1	-	0.4962
Artesunate	154	39	25.32	(18.67, 32.95)	1.14	(0.78, 1.68)	
Total	1487	344	23.13	(21.01, 25.36)	-	-	-

Table 3
Incidence of post-malarial anemia within 28 days after discharge.

Treatment	Clinical malaria admissions	Delayed anemia episodes	Time At Risk (CMAR)	Rate estimations		Model estimations		
				Incidence Rate (Episodes per 1000 CMAR)	95% Confidence Interval	Hazard Ratio	95% Confidence Interval	p-value
Quinine	1333	305	1068.62	285.42	(255.12, 319.31)	1	-	0.4879
Artesunate	154	39	122.34	318.8	(232.92, 436.33)	1.12	(0.81, 1.57)	
Total	1487	344	1190.95	288.84	(259.88, 321.04)	-	-	-

CMAR: children month at risk

Patients at risk (deaths / episodes)
Quinine 1333 (0 / 38) 1295 (1 / 64) 1230 (3 / 86) 1141 (2 / 117) 1022
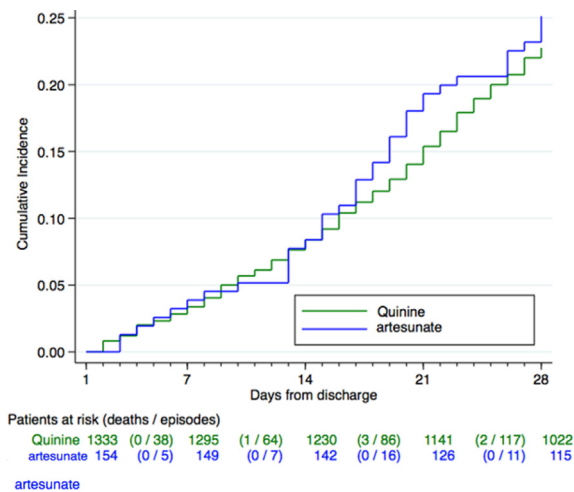artesunate 154 (0 / 5) 149 (0 / 7) 142 (0 / 16) 126 (0 / 11) 115

Figure 2. Weighted log-rank test for the Cumulative Incidence of anaemia episodes: Chi2 = 0.48; p-value = 0.4877.
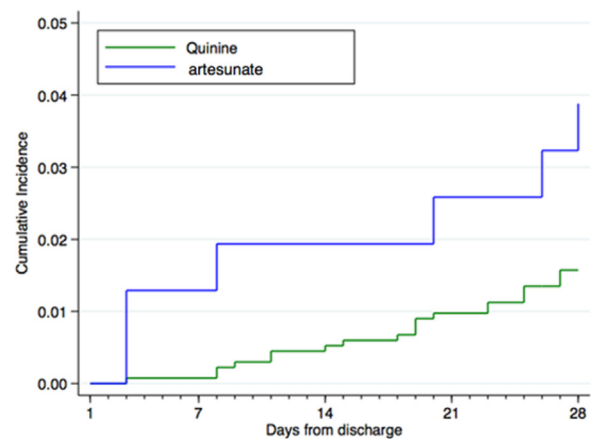

Figure 3. Weighted log-rank test for the Cumulative Incidence of blood transfusions: Chi2(1) = 4.23; p-value = 0.0398.

Figure 3 shows the cumulative incidence curve estimates for blood transfusions by treatment group (Weighted log-rank test for the Cumulative Incidence of blood transfusions: Chi2 = 4.23; p-value = 0.0398).

Discussion

The overall prevalence of post-malarial anemia observed in the study was 23.13%, with an estimated incidence rate of 288.84 episodes/1,000 CMAR in the follow-up period (28 days after discharge). There were no differences between treatment groups, although the study showed a higher association of blood transfusion treatment with artesunate.

Rolling et al. (Rolling et al., 2014) conducted a prospective observational study to investigate delayed hemolysis in African children with severe malaria treated with parenteral artesunate and found a percentage of delayed hemolysis of 7% in a cohort of 72 children (7). Children who presented with delayed hemolysis were younger and had higher mean parasitemia than those without it. In a recent update of this study, Rolling et al. (Scheu et al., 2019) found a slightly lower percentage (5%) and stated that PADH and hyperparasitemia were associated with early malarial anemia (Scheu et al., 2019). However, Rolling et al. used a restrictive definition of delayed hemolysis, taking into account levels of hemoglobin, lactate dehydrogenase, and haptoglobin during an active follow-up. This may explain the differences with the current analysis, which was based on passive detection, whereby only levels of hematocrit were evaluated without using specific markers of hemolysis. A prospective study developed in The Democratic Republic of the Congo (Burri et al., 2014) in patients treated with parenteral artesunate also demonstrated a decrease in hemoglobin levels between days seven and 21 after treatment in 11.4% of patients. Only 1% of cases presented severe anemia during follow-up. All delayed anemia cases were clinically manageable and

evolved without complications. Another recent study in 91 Ugandan children with severe malaria treated with parenteral artesunate found that none of those patients met their standardized definition of PADH (Hawkes et al., 2019) and, like others, anemia was prevalent on admission. The abovementioned studies lacked a control group, and the criteria used to investigate anemia hindered the extrapolation and comparison with the results from the current analysis.

An open-label, randomized controlled trial study conducted in The Democratic Republic of Congo by Fanello et al. (Fanello et al., 2017) compared the proportion of children with a $\geq 10\%$ reduction in hemoglobin during the six weeks after treatment with quinine or parenteral artesunate and was able to demonstrate that up to 5% of the patients in each group presented a delayed anemia episode. The authors could not show any statistically significant differences between groups of treatment (Fanello et al., 2017). In a meta-analysis performed pooling data from a variety of clinical trials for the treatment of uncomplicated malaria with oral artemisinin derivatives, no association was found between the use of oral artemisinin-based therapies and the incidence of delayed anemia (defined as anemia -severe or not- observed any time of follow-up from day seven to day 28) although a significant drop of haemoglobin on day seven after treatment was detected (Sagara et al., 2014).

This study found a higher association of blood transfusions with the use of artesunate treatment. One possible explanation could be the decreases in malaria transmission and the associated changes in severe malaria presentation with older and less immune children in the artesunate period. Also, a less conservative use of blood products could have influenced these differences.

This study has several important limitations. First, most of the children included in the analysis received quinine in comparison to artesunate. This difference in the number of treatments may be explained for different reasons, including a decrease of malaria

Table 4
Incidence of blood transfusions within 28 days after discharge.

Treatment	Clinical malaria admissions	Blood transfusions	Time at Risk (CMAR)	Rate estimations		Model estimations		
				Incidence Rate (Episodes per 1000 CMAR)	95% ConfidenceInterval	Hazard Ratio	95% Confidence Interval	p-value
Quinine	1333	21	1174.18	17.88	(11.66, 27.43)	1	-	-
Artesunate	154	6	134.14	44.73	(20.09, 99.56)	2.51	(1.01, 6.21)	0.0471
Total	1487	27	1308.32	20.64	(14.15, 30.09)	-	-	-

CMAR: children month at risk

cases, more rational management of those cases -including severe ones- or a more extended period of quinine use.

Second, the study used hematocrit to define anemia, and it is well known that PCV may not accurately represent hemoglobin levels and the degree of anemia (Quinto et al., 2006). Third, more detailed data on other common causes of anemia such as iron deficiency, hemoglobinopathies, bacteremia, viral infections (Parvovirus B19, Epstein Barr Virus, HIV) or intestinal parasitic infections, which could have confounded the relation studied, were not systematically available, and therefore the post-malarial anemia is likely an overestimation (Moraleda et al., 2017). Besides, the absence of systematic follow-up of all discharged alive patients undoubtedly has contributed to missing a significant number of cases. This low follow-up rate is a good reason for caution in the generalization and extrapolation of the results. Finally, the relatively small sample size for the artesunate component (only 129 children treated with artesunate with a measure of their potential anemia available in the following 28 days) and the low number of anemia cases, may have impaired the statistical power of some of the comparisons; larger sample sizes could have helped to provide more conclusive evidence.

Conclusions

This study found a high overall frequency of post-malarial anemia in children in Mozambique but was unable to find differences in children treated with either intravenous quinine or parenteral artesunate. The high burden described in this study irrespective of the treatment received, and the high prevailing risk of multifactorial anemia in this setting, urgently call for the establishment of an active follow-up system to ensure the well-being of the ones who survive their malarial episodes.

Authors' contribution

RV, LM, and QB conceived the study and contributed to the design. LQ, QB, and RV analyzed and interpreted the data. RV wrote the first draft of the manuscript, together with QB. All authors critically revised and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed in the current study are available from the corresponding author on reasonable request.

Ethical approval and consent to participate

This study retrospectively assessed data collected in the context of routine clinical practice. The MSS in place at MDH has been approved by the National Bioethics Committee for Health of Mozambican (CNBS-IRB00002657). The analytical plan of this specific analysis was assessed and approved by CISM's Internal Scientific Committee.

Consent for publication

All the authors have read and approved the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.05.089.

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