

Diagnosis, Clinical Presentation, and In-Hospital Mortality of Severe Malaria in HIV-Coinfected Children and Adults in Mozambique

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Background. Severe falciparum malaria with human immunodeficiency virus (HIV) coinfection is common in settings with a high prevalence of both diseases, but there is little information on whether HIV affects the clinical presentation and outcome of severe malaria.

Methods. HIV status was assessed prospectively in hospitalized parasitemic adults and children with severe malaria in Beira, Mozambique, as part of a clinical trial comparing parenteral artesunate versus quinine (ISRCTN50258054). Clinical signs, comorbidity, complications, and disease outcome were compared according to HIV status.

Results. HIV-1 seroprevalence was 11% (74/655) in children under 15 years and 72% (49/68) in adults with severe malaria. Children with HIV coinfection presented with more severe acidosis, anemia, and respiratory distress, and higher peripheral blood parasitemia and plasma *Plasmodium falciparum* histidine-rich protein-2 (PfHRP2). During hospitalization, deterioration in coma score, convulsions, respiratory distress, and pneumonia were more common in HIV-coinfected children, and mortality was 26% (19/74) versus 9% (53/581) in uninfected children ($P < .001$). In an age- and antimalarial treatment-adjusted logistic regression model, significant, independent predictors for death were renal impairment, acidosis, parasitemia, and plasma PfHRP2 concentration.

Conclusions. Severe malaria in HIV-coinfected patients presents with higher parasite burden, more complications, and comorbidity, and carries a higher case fatality rate. Early identification of HIV coinfection is important for the clinical management of severe malaria.

Severe malaria and human immunodeficiency virus (HIV) coinfection are common in settings with both

high malaria transmission intensity and high HIV-1 seroprevalence [1–3]. Various interactions between malaria and HIV have been described, and the diseases negatively affect their reciprocal courses [4, 5]. HIV transmission and progression may be accelerated by malaria [6–8]. Conversely, HIV infection increases the incidence of clinical malaria, severe malaria, and malaria-related mortality, particularly in adults with deteriorating immune status [9–13]. However, only few studies address the effects of HIV infection on severe malaria morbidity and mortality in African children, who carry the highest burden of disease [2, 14–18]. We studied the effects of HIV-1 coinfection on diagnosis, clinical presentation, and outcome of patients with

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severe malaria admitted in a tertiary referral hospital in Beira, Mozambique, an area with both a high prevalence of HIV infection as well as malaria.

METHODS

This study was part of a large multinational trial comparing artesunate versus quinine in the treatment of severe falciparum malaria in African children (AQUAMAT, registration number ISRCTN50258054), the results of which have been published elsewhere [19]. This substudy was conducted at Hospital Central da Beira (HCB), Beira, Mozambique, between October 2005 and July 2010. HCB is an 800-bed tertiary referral hospital in the port city of Beira, which is built along the swampy grounds at the mouth of the Pungwe River where malaria transmission is perennial and malaria is meso-endemic [20]. The HIV burden in the city of Beira is reflected in the HIV prevalence in pregnant women, reported as approximately 30% in 2004 [21].

Ethics approval, including confidential HIV and CD4⁺ testing, was granted by the Comité Nacional de Bioética para a Saúde in Mozambique and the Oxford Tropical Research Ethics Committee.

Children (<15 years) and adults (≥15 years) presenting with suspected severe malaria according to modified World Health Organization (WHO) clinical criteria were screened using a *Plasmodium* lactate dehydrogenase (pLDH)-based and *Plasmodium falciparum* histidine-rich protein-2 (PfHRP2)-based rapid diagnostic test (RDT) and a peripheral blood slide [22, 23]. Patients with a positive pLDH-based RDT were included in the trial, provided that full written informed consent was given by the patient or carer. Severity criteria included decreased consciousness (coma or severe prostration), convulsions, respiratory distress or acidotic breathing, shock, severe symptomatic anemia (<5 g/dL), hypoglycemia (<3 mmol/L), hemoglobinuria, severe jaundice, or a convincing history of anuria or oliguria in adult patients. Patients were excluded if treated with a parenteral antimalarial >24 hours before admission. Patients were randomized to treatment with either parenteral artesunate or quinine.

A venous blood sample was taken for peripheral blood parasite counts, hematocrit (Hct), quantitative assessment of plasma PfHRP2 (a marker of total body parasite burden) [24, 25], biochemistry and acid-base parameters (EC8⁺ cartridge for i-STAT handheld analyzer), as well as HIV testing and CD4⁺ lymphocyte count. HIV antibody testing was performed according to the sequential test algorithm by the Mozambican HIV testing guidelines, comprising of a screening test (Determine HIV-1/2, Abbott Laboratories, Abbott Park, IL) followed by a confirmation test (Uni-Gold HIV, Trinity Biotech PLC,

Bray, Ireland) in case of a positive screening result. Clinical staff and patients did not have access to the HIV-1 test results and laboratory staff did not have access to patient data. Surviving patients or their guardians received voluntary counseling and testing after recovery, and if indicated, were offered further treatment and follow-up according to national guidelines. HIV data were linked to the main database through an anonymized study number.

Quality assessment of slide reading was performed at the Mahidol-Oxford Tropical Medicine Research Unit in Bangkok on 1348/2017 (67%) of screening slides and 100% of slides of enrolled patients, and was used as reference. Quantitative parasite counts were calculated from thin film (count per μL = count per 1000 red blood cells \times 125.6 \times Hct) assuming an MCV of 80 fL [26] or thick film (count per μL = count per 200 white blood cells [WBC] \times 40) assuming a WBC of 8000/ μL [26, 27].

Statistical Analysis

Data were analyzed with STATA, version 12 (StataCorp, College Station, TX). Categorical variables were compared between HIV-negative and HIV-positive cases with χ^2 or Fisher's exact test. Normally distributed or \log^{10} -normalized variables were compared using a Student *t* test, the remainder using Mann-Whitney *U* test. Weight-for-age *Z* scores as a measure of malnutrition were calculated using STATA applications based on the WHO Child Growth Standards [28, 29]. "Strictly" defined severe malaria was based on modified WHO criteria [19, 22]. In HIV-positive patients, CD4⁺ lymphocyte count or percentage-based immunological staging (not significant, mild, advanced, severe) was performed according to the WHO classification [30].

To determine the prognostic significance of HIV coinfection or the WHO HIV immunological stage classification, a logistic regression model was constructed with death as the dependant variable and HIV infection in addition to established predictors of death as the independent variables, including coma, prostration, convulsions, hypoglycemia, respiratory distress, shock, hemoglobin (g/dL), base excess (BE; mmol/L), \log^{10} blood urea nitrogen (mg/dL), \log^{10} parasitemia (parasites/ μL), plasma PfHRP2 [as \log^{10} and (\log^{10})², ng/mL] [19, 24, 25, 31–33] and weight-for-age *Z* scores [34]. Since the case fatality rates between children and adults differed significantly ($P < .0001$) and the number of adult participants was limited, only children were included in the logistic regression model, which was adjusted for age and antimalarial treatment (artesunate or quinine) [19, 35]. Using a stepwise approach, only covariates that were significant at $P < .05$ were retained in the final model. The a priori-specified interaction between HIV infection and PfHRP2 was also assessed.

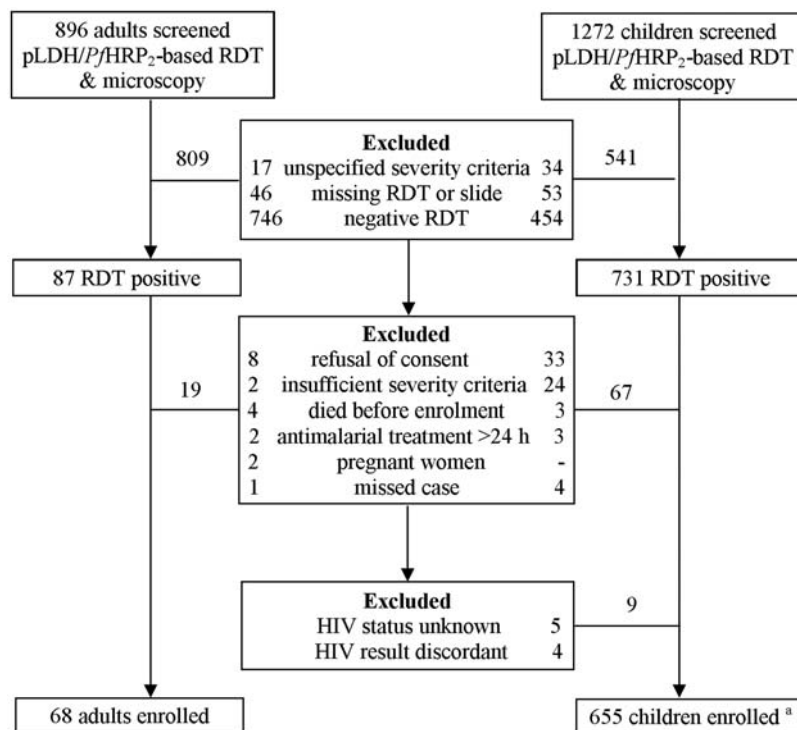
RESULTS

Between October 2005 and July 2010, 896 adults were screened for severe malaria, out of whom 87 (9.7%) had a positive pLDH-based RDT, an inclusion criterion for the treatment trial (Figure 1). Out of these, 68 adults were enrolled in the treatment trial. In 1272 children with suspected severe malaria, 731 or 57.5% (724 positive pLDH and *Pf*HRP2 tests, 7 with positive *Pf*HRP2-based test only) had a positive RDT of which 655 were enrolled. In screened adult patients with quality assessment of the peripheral blood slide (n = 513), the sensitivity of the *Pf*HRP2-based RDT compared to expert microscopy was 44.3% and the specificity was 95.2%. For the pLDH-based RDT, sensitivity was 38.8% and specificity 98.5%. In children (n = 835), the sensitivities of these RDTs were 92.6% and 85.3%, and the specificities were 64.5% and 83.3%, respectively. The combination of a negative RDT and a positive malaria slide was associated with low parasitemia. The parasite density (geometric mean; 95% confidence interval [CI]) with a negative *Pf*HRP2-based test and positive slide with reported parasite density was 232 (141–383) parasites/ μ L

in adults (n = 89) and 180 (90–359) parasites/ μ L in children (n = 40; $P = .56$).

HIV status was assessed in 727/732 (99%) patients with severe malaria. Patients with discordant HIV antibody test results (n = 4) were excluded from the analysis. Four children aged <18 months were classified as HIV positive on the basis of their serostatus in absence of viral load testing and were included. HIV-1 seroprevalence was 74/655 (11%) in children and 49/68 (72%) in adults.

Baseline characteristics are shown in Table 1. HIV-positive status in coinfecting patients was reported on admission in 9/74 (12%) of children and 5/49 (10%) of adults, of which 5 and 4 were on antiretroviral treatment, respectively. By history of the carer, 10/74 (14%) of HIV-coinfecting children had a prior chronic illness (6 tuberculosis, 2 tuberculosis with chronic otitis media, 2 chronic otitis media), versus 20/579 (3%) of HIV-uninfected children (8 tuberculosis, 1 sequelae of previous cerebral malaria, 4 congenital heart disease, 1 chronic otitis media, 6 bronchial asthma, 2 no information; $P < .001$). In HIV-coinfecting adults, 11/49 (22%) had a history of chronic disease (5 tuberculosis, 1 hemiparesis after stroke, 1



^aSeven children were enrolled based on a positive Paracheck test only.

Figure 1. Study profile. Abbreviations: HIV, human immunodeficiency virus; *Pf*HRP2, *Plasmodium falciparum* histidine-rich protein-2; pLDH, *Plasmodium* lactate dehydrogenase; RDT, rapid diagnostic test.

Table 1. Baseline Characteristics of Children and Adults With Severe Malaria According to HIV Status

Characteristic	Children <15 Years			Adults ≥15 Years		
	HIV Negative (n = 581)	HIV Positive (n = 74)	P Value	HIV Negative (n = 19)	HIV Positive (n = 49)	P Value
Female	279 (48%)	33 (45%)	.58	7 (37%)	22 (45%)	.55
Age, years (median, IQR)	3.6 (2.5–5.0)	5.0 (3.0–8.0)	.0001	18 (16–23)	27 25–38	.0002
Presenting symptoms						
Coma	441 (76%)	54 (73%)	.58	13 (68%)	35 (71%)	.81
Convulsions	506 (87%)	56 (76%)	.008	10 (53%)	18 (37%)	.23
Prostration	132 (23%)	19 (26%)	.57	5 (26%)	12 (24%)	1.0
Shock ^a	21 (4%)	3 (4%)	.74	0 (0%)	3 (6%)	.55
Severe respiratory distress	37 (6%)	14 (19%)	<.001	1 (5%)	6 (12%)	.66
Severe acidosis (BE ≤−8 mmol/L)	108 (22%)	26 (38%)	.004	1 (8%)	12 (27%)	.26
Hypoglycemia	32 (6%)	8 (11%)	.07	1 (5%)	2 (4%)	1.0
Severe anemia with respiratory distress	67 (12%)	15 (21%)	.028	0 (0%)	3 (6%)	.55
Black water fever	24 (4%)	5 (7%)	.36	4 (21%)	10 (20%)	1.0
Severe jaundice	15 (3%)	5 (7%)	.06	2 (11%)	4 (8%)	1.0
Anuria/oliguria ^b	0 (0%)	2 (4%)	1.0
Hyperparasitemia (>10%)	106 (21%)	21 (32%)	.043	3 (21%)	12 (29%)	.74
“Strictly defined” severe malaria ^c	533 (94%)	66 (89%)	.08	16 (94%)	41 (89%)	1.0

Abbreviations: BE, base excess; HIV, human immunodeficiency virus; IQR, interquartile range.

^a For children: compensated and decompensated shock combined. In adults: only decompensated shock.

^b In adults only.

^c Based on WHO criteria [22].

epilepsy, 1 hepatitis, 3 herpes zoster) versus 1/16 (6%) (hypertension, 3 no information; $P = .108$).

Parasitological Markers in Pediatric Cases

Expert slide readings with quantitative results were available from 575/655 (88%) of enrolled children with known HIV status. Geometric mean (95% CI) parasitemia was 47 140 (37 988–58 498) in HIV-negative ($n = 510$) and 67 977 (37 143–124 408) in HIV-positive children ($n = 65$; $P = .26$). Geometric mean (95% CI) plasma PfHRP2 concentration assessed in 653/655 (>99%) children was 831 (707–975) ng/mL in HIV-negative ($n = 510$) and 1395 (911–2136) ng/mL in coinfecting children ($n = 65$; $P = .0321$; Figure 2).

Clinical Manifestations of Severe Malaria According to HIV Status

HIV-coinfecting children were older and differed in their clinical presentation from uninfected children with an increased frequency of severe acidosis and severe anemia (both clinically and laboratory assessed) and respiratory distress (Tables 1 and 2). Blood urea nitrogen (BUN) concentrations were also higher in HIV-infected children. On physical examination, HIV-positive children had significantly lower weight-for-age Z scores and more frequent oral candidiasis and

lymphadenopathy. In the adult patients, similar nonsignificant trends were recorded.

CD4⁺ percentages and/or absolute counts were available for 68/74 (92%) of HIV-positive children. Increasing HIV-associated immunodeficiency according to the WHO classification was associated with increasing plasma PfHRP2 concentration (nonparametric test for trend; $P = .022$) but not with mortality ($P = .23$).

Comorbidities, Complications, and Outcome in Pediatric Cases

Comorbidities during hospitalization were more common in HIV-coinfecting children (15/74 [20%]) compared with HIV-negative children (51/581 [9%]; $P = .002$). Pneumonia was suspected clinically in 19 (3%) of HIV-negative children versus 9 (12%) in HIV-positive children ($P < .001$). Chest X-rays were sparsely available, and only a minority of cases had a radiologically confirmed diagnosis of pneumonia. Although culture facilities were lacking, clinical sepsis was more common in HIV-coinfecting children as well as a variety of other comorbidities (Table 3). After admission, HIV-coinfecting children developed more severe malaria related complications compared to HIV-negative children (Table 3). HIV-coinfecting children received more blood transfusions (38/74 [51%] vs

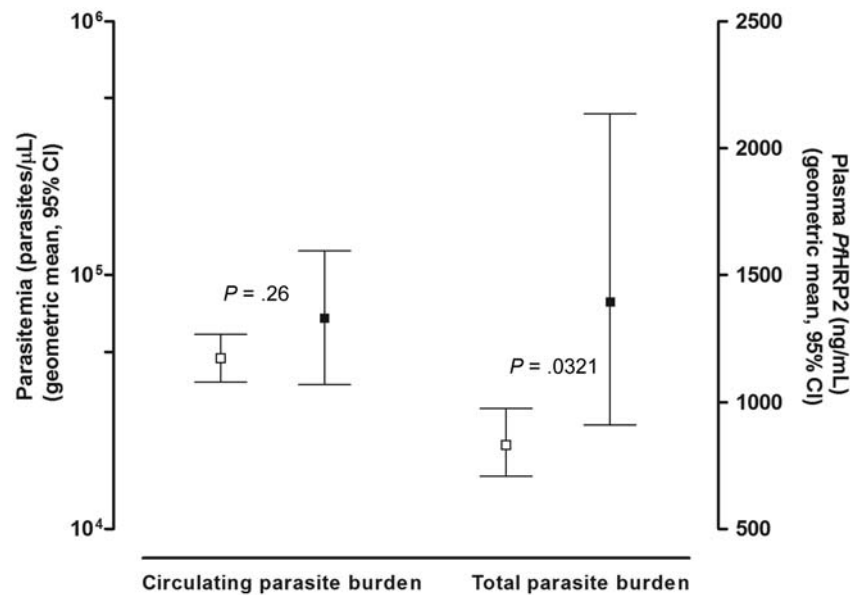


Figure 2. Comparison of the circulating peripheral blood parasitemia (left y-axis) and plasma *PfHRP2* concentration as a measure of the total body parasite burden (right y-axis), between human immunodeficiency virus (HIV)–negative (□, n = 510) and HIV-positive (■, n = 65) children with quantified plasma *PfHRP2* and peripheral blood parasitemia. Abbreviations: CI, confidence interval; *PfHRP2*, *Plasmodium falciparum* histidine-rich protein-2.

224/581 [39%]; $P = .034$) and antibiotics (65/74 [88%] vs 447/581 [77%]; $P = .033$) in HIV-negative children.

In surviving children without neurological sequelae, median hospitalization time (interquartile range [IQR]) was 5 (4–7) days in HIV-coinfected versus 4 days (3–5) in HIV-negative children ($P = .0012$). The mortality in HIV-coinfected children was 19/74 (26%) versus 53/581 (9%) in HIV-uninfected children ($P < .001$). This difference remained significant when only patients with “strictly” defined severe malaria (see the “Methods” section) were included in the analysis. Although this substudy was not powered to look at the treatment effect of artesunate versus quinine, the mortality in HIV-positive children treated with artesunate was 22.2% versus 31% with quinine (odds ratio [OR] (95% CI) 0.63 [.22–1.85]; $P = .40$) and in HIV-negative children 8.1% versus 10.1% (OR for artesunate 0.78 [0.44–1.38]; $P = .39$). Stratification according to concomitant antiretroviral treatment (n = 5) or antituberculosis treatment (n = 5) did not alter any of the results (data not shown).

In a logistic regression model adjusted for age and antimalarial treatment, with death as dependent variable and established predictors of severe malaria as independent variables, significant predictors in the final model (n = 482) were renal impairment (\log^{10} BUN), acidosis (BE), parasitemia (\log^{10} parasitemia; but an inverse correlation), and plasma *PfHRP2* concentration (Table 4). HIV infection was correlated with increased mortality (unadjusted OR 3.44; 95% CI 1.88–6.28),

but was not an independent prognosticator when plasma *PfHRP2* was introduced into the model, which was related to the correlation between HIV status and plasma *PfHRP2* (test for trend across ordered groups; $P < .0001$). The independent predictors were identical in a model including categorical HIV immunological stages rather than presence of HIV infection as a binary variable. Also, HIV status did not contribute significantly to the final model when introduced as an interaction term with plasma *PfHRP2* concentration.

DISCUSSION

This is the first prospective study to report the different clinical presentations of severe malaria, parasite burden, and mortality in HIV-coinfected patients. It was shown that HIV-coinfected children with severe malaria were more undernourished and presented more frequently with severe acidosis, severe anemia, respiratory distress, and elevated BUN concentrations, and similar (albeit nonsignificant) trends were found in HIV-infected adults. Previous studies carried out in areas of high malaria transmission have reported increased prevalence and severity of severe anemia and a higher 7-, 28-, or 90-day post-admission mortality in HIV-coinfected children without differences in admission parasitemia [15, 16, 36]. In addition to these findings, we established that a metabolic acidosis is more frequent in HIV-coinfected children. Acidosis in severe malaria has been associated with severe anemia and

Table 2. Clinical Examination and Laboratory Assessments in Patients With Severe Malaria According to HIV Status

Assessment	Children <15 Years			Adults ≥15 Years		
	HIV Negative (n = 581)	HIV Positive (n = 74)	P Value	HIV Negative (n = 19)	HIV Positive (n = 49)	P Value
Clinical examination						
Weight-for-age Z score ^a	-1.0 (1.3)	-1.6 (1.1)	.0001
Temperature (°C)	38.2 (0.9)	38.3 (1.0)	.76	38.1 (1.2)	38.2 (1.3)	.76
Blood pressure (mmHg)						
Systolic	103 (16)	104 (16)	.48	112 (16)	113 (22)	.82
Diastolic	64 (13)	65 (16)	.65	69 (14)	69 (13)	.89
Respiratory rate (breaths/min)	39 (10)	39 (9)	.98	26 (5)	29 (8)	.14
Coma depth (N, median, range)						
BCS	70, 2 (0–5)	7, 2 (2–3)	.29
GCS	511, 8 (3–15)	67, 9 (3–15)	.287	18, 10 (3–14)	48, 8 (3–15)	.54
Oral candidiasis	1 (<1%)	9 (12%)	<.001	0	4 (9%)	.57
Lymphadenopathy	16 (3%)	20 (27%)	<.001	1 (6%)	5 (10%)	.54
Laboratory assessments						
BUN (mg/dL) ^b	12 (11–12)	16 (13–19)	<.0001	23 (15–35)	32 (25–40)	.18
Hemoglobin (g/dL) ^c	8.2 (2.7)	7.5 (2.7)	.033	11.3 (2.6)	10.2 (3.2)	.16
pH ^d	7.40 (0.11)	7.38 (0.14)	.26	7.39 (0.09)	7.37 (0.13)	.64
HCO ₃ (mmol/L) ^e	19.9 (4.9)	17.7 (6.6)	.0267	21.8 (3.1)	19.7 (5.7)	.17
Base excess (mmol/L) ^f	-5 (6)	-7 (8)	.0461	-3 (4)	-6 (8)	.43
Slide <i>Pf</i> positive	547 (99%)	72 (99%)	1.0	18 (100%)	45 (96%)	1.0
Parasitemia (parasites/μL) ^g	47 141	68 320	.25	133 653	61 525	.36
Geometric mean (95% CI)	(38 005–58 474)	(37 680–123 874)		(59 082–302 343)	(24 628–153 704)	
<i>Pf</i> HRP2 (ng/mL) ^h	834	1452	.0197	457	2471	.0072
Geometric mean (95% CI)	(712–977)	(983–2145)		(93–2236)	(1509–4047)	
CD4 ⁺ percentage ⁱ	...	21 (12–27)	13 (6–26)	...
CD4 ⁺ absolute cell count ^j	...	235 (118–429)	199 (76–405)	...
HIV-associated immunodeficiency^k						
Not significant	...	16 (23%)	6 (14%)
Mild	...	13 (19%)	7 (16%)
Advanced	...	12 (18%)	8 (19%)
Severe	...	27 (40%)	22 (51%)

Data are No. (%), mean (SD), or median (IQR), unless otherwise indicated.

Abbreviations: BCS, Blantyre coma score; BUN, blood urea nitrogen; CI, confidence interval; GCS, Glasgow coma score; HIV, human immunodeficiency virus; IQR, interquartile range; *Pf*HRP2, *Plasmodium falciparum* histidine-rich protein-2.

^a For children ≤10 years (HIV-negative children, n = 563; HIV-positive children, n = 67).

^b BUN was available in n = 487 and n = 67 children, n = 15 and n = 45 adults, respectively.

^c Hemoglobin was available in n = 551 and n = 70 children, n = 19 and n = 48 adults, respectively.

^d pH was available in n = 489 and n = 68 children, n = 13 and n = 45 adults, respectively.

^e HCO₃ was available in n = 490 and n = 68 children, n = 13 and n = 44 adults, respectively.

^f BE was available in n = 488 and n = 68 children, n = 13 and n = 45 adults, respectively.

^g Parasitemia reported from all patients with peripheral blood slide count, n = 511 and n = 66 children, n = 14 and n = 44 adults, respectively.

^h *Pf*HRP2 reported for all patients with *Pf*HRP2 sample n = 580 and n = 73 children, n = 18 and n = 48 adults, respectively.

ⁱ CD4⁺ percentage reported for HIV-infected children <5 years (n = 34) and adults (n = 42).

^j CD4⁺ absolute cell counts reported for HIV-infected children ≥5 years (n = 34) and adults (n = 43).

^k Based on WHO criteria [30].

respiratory distress or deep breathing and is an established strong predictor of mortality in adult as well as pediatric severe malaria [31, 37]. Total body parasite burden, measured as plasma *Pf*HRP2 concentrations [24], was higher in

HIV-infected children and positively correlated with the severity of immunosuppression according to WHO immunological classification. This total parasite burden includes the sequestered parasite burden, which causes impaired

Table 3. Comorbidity, Complications, and Outcome According to HIV Status

	Children <15 Years			Adults ≥15 Years		
	HIV Negative (n = 581)	HIV Positive (n = 74)	P Value	HIV Negative (n = 19)	HIV Positive (n = 49)	P Value
Comorbidity						
Suspected pneumonia	19 (3%)	9 (12%)	<.001	1 (5%)	2 (4%)	1.0
Confirmed by CXR	5 (26%)	4 (44%)	.41	1 (100%)	0 (0%)	.3
Clinical sepsis ^a	9 (2%)	4 (5%)	.049	0 (0%)	1 (2%)	1.0
Suspected meningitis ^a	3 (<1%)	1 (1%)	.38	1 (5%)	0	1.0
Gastroenteritis ^b	7 (1%)	2 (3%)	.27	0	0	...
Other significant comorbidities ^c	22 (4%)	7 (9%)	.025	0	0	...
Complications (not present on admission)						
Development of coma	3 (<1%)	2 (3%)	.101	1 (5%)	2 (4%)	1.0
Deterioration coma score	22 (4%)	9 (12%)	.001	2 (11%)	6 (12%)	1.0
Convulsions developing or persisting >6 hours after admission	61 (11%)	18 (24%)	.001	2 (11%)	3 (6%)	.61
Respiratory distress	6 (1%)	7 (9%)	<.001	4 (21%)	5 (10%)	.25
Severe anemia (<5 g/dL)	12 (2%)	2 (3%)	.67	0	1 (2%)	1.0
Black water fever	13 (2%)	4 (5%)	.11	0	2 (4%)	1.0
Renal failure ^d	3 (<1%)	2 (3%)	.10	3 (16%)	8 (16%)	1.0
Outcome						
Mortality	53 (9%)	19 (26%)	<.001	4 (21%)	17 (35%)	.38
Mortality in "strictly" defined severe malaria	53/533 (10%)	19/66 (29%)	<.001	4/16 (25%)	17/41 (41%)	.36
Neurological sequelae at 28 days	6 (1%)	2 (3%)	.23	0	0	...

Abbreviations: CXR, chest X-ray; HIV, human immunodeficiency virus.

^a No culture facilities available.

^b Defined as >6 loose stools/24 h.

^c Other significant comorbidities in HIV-negative children included: suspected intoxication with traditional medicine, suspected hepatitis, burn of hand (1%), asthma/bronchitis, undefined skin rash, reactive arthritis, parasitosis (tungiasis, ascariis), herpes simplex virus labial ulcers, acute otitis media, tonsillitis, conjunctivitis, fever of unknown origin, suspected encephalitis; in HIV-positive children: suspected intoxication with traditional medicine, asthma/bronchitis, impetigo, submandibular abscess, keratitis, suspected encephalitis.

^d Defined as urine output <0.5 mL/kg/h for >24 h or blood urea nitrogen >60 mg/dL.

microcirculatory flow, an important cause of metabolic acidosis in severe malaria [24, 25].

In line with other studies, HIV-coinfected children with severe malaria were older [2, 14, 16] and had higher parasite densities [14, 38]. These results suggest that failure of the acquired immunity in HIV infection leads to a decreased ability to control parasitemia, which increases the risk of developing severe malaria with associated high mortality [3, 9, 10, 39]. The clinical presentation of malaria in HIV-coinfected children depends on malaria specific immunity, which varies according to age and malaria transmission intensity [40, 41] as well as HIV-related immunosuppression [9, 11, 14, 39]. Although our study design did not allow such comparison, our findings support the hypothesis that HIV-related immunosuppression increases the risk of severe malaria in adults, since the HIV-1 seroprevalence in severe malaria was more than twice the reported the HIV-1 seroprevalence in the adult population [42].

Elevated BUN concentrations have been reported in HIV-coinfected adults with severe malaria [11, 43], and renal failure due to malaria-induced acute tubular necrosis has been described as a common complication and cause of death in Asian adults [44, 45]. It has been hypothesized that HIV-coinfected adults are more likely to have an HIV-mediated impaired renal function, although normalization of renal function following resolution of the malaria episode has also been described [11]. The significance of elevated BUN concentrations in HIV-positive patients, particularly in children, and its association with mortality as also observed in other studies [32, 33] requires further investigation.

In the adults, sensitivity of the RDTs for diagnosing malaria was remarkably low (<50%). This was explained by low parasite densities on the peripheral blood slide, below the level of detection of the tests [23]. Patients with a negative PfHRP2-based RDT and low peripheral blood parasitemia include

Table 4. Logistic Regression Analysis for Children, Adjusted for Age, Showing the Prognostic Value of Significant Risk Factors Assessed on Admission for In-Hospital Survival of Children With Severe *Falciparum* Malaria

Variable	Odds Ratio (95% CI)	P Value
Plasma base excess	0.89 (.84–.94)	<.001
[log] Blood urea nitrogen	3.81 (1.90–7.65)	<.001
[log] Parasitemia	0.66 (.47–.89)	.007
[log] Plasma PfHRP2 (squared)	1.47 (1.10–1.95)	.008
[log] Plasma PfHRP2	0.12 (.03–.48)	.003
Antimalarial drug (artesunate vs quinine)	0.37 (.18–.78)	.009

The association between death and [log] plasma PfHRP2 was U-shaped and best described using a quadratic function. The patients with low PfHRP2 concentrations signify children with a low parasite burden where severe illness is likely caused by an alternative diagnosis than severe malaria (Hendriksen et al. *PLoS Med*, in press). Introduction of an interaction term (HIV-1 status × plasma PfHRP2) did not improve the model ($P = .88$). $R^2 = 0.29$ for the final model ($n = 482$).

Abbreviations: CI, confidence interval; HIV, human immunodeficiency virus; PfHRP2, *Plasmodium falciparum* histidine-rich protein-2.

those with very low total parasite burdens, where an alternative diagnosis other than malaria is more likely [9]. Unfortunately, in the setting of the study, possibilities for establishing accurate alternative diagnoses other than malaria were limited. The Mozambican national malaria control program recommends parasitological diagnosis of malaria in adults by rapid diagnostic test, although in referral hospitals, severe malaria is commonly diagnosed by peripheral blood slide [42]. Slide-positive RDT-negative severe illness represents patients with low parasitemia warrants diagnosis and treatment of other, possibly HIV-related, alternative illnesses [14, 46, 47]. Further studies to assess the diagnostic work-up and management of this patient group are needed.

Comorbidities were more frequent in HIV-coinfected children than in HIV-uninfected children; notably suspected pneumonia and sepsis. In the AQUAMAT trial site in Muheza, Tanzania, where blood cultures and confidential HIV testing were performed, 8/38 (21%) of HIV-coinfected children had a positive blood culture versus 45/855 (5%) in HIV-negative children ($P < .001$) with an almost 3-fold increased case fatality rate (14/38 (37%) in HIV-coinfected children versus 112/855 (13%) in HIV-uninfected children ($P < .001$) [unpublished data; personal communication, Ilse Hendriksen]. Pediatric severe malaria with HIV coinfection has been associated with an increased risk of non-*typhi* *Salmonella* (NTS) [2] and/or gram-negative bacteremia, both leading to an increased risk of death [14]. A study in southern Mozambique (an area with similar high HIV prevalence) reported an incidence of 5.4% of concomitant bacteremia in pediatric severe malaria with *Streptococcus pneumoniae* being the most frequently identified

organism, especially in children with respiratory distress, and was associated with a higher case fatality [48].

Case fatality with HIV coinfection was 282% higher in children ($P < .001$) and 64% higher in adults with severe malaria ($P = .28$). However, in a logistic regression model, HIV infection was not an independent predictor of death when plasma PfHRP2 was included in the regression model, whereas there was a clear correlation between HIV status and plasma PfHRP2 concentration, which is a measure of the total body parasite burden, including the sequestered parasites [24, 25]. This again suggests that HIV-induced immune incompetence compromises control of the malaria parasite burden and thus severity of the infection. It also suggests that this mechanism is more important than HIV-related comorbidity, and underscores the importance of potent antimalarial treatment in these children with parenteral artesunate [19].

In accordance with data from Asian settings [35], convulsions, hypoglycemia, and symptomatic severe anemia were more frequent in children, whereas renal impairment and severe jaundice were more common in adult patients in the current African study. Acidosis and coma were prominent in both groups, whereas shock was rare.

The main limitations of this study include the lack of diagnostic information to assess the additional pathology responsible for increased severe malaria mortality in HIV-coinfected patients. Chest X-rays were not routinely performed and blood culture facilities were unavailable at the time of this study. In addition, clinical malaria may lower the CD4⁺ lymphocyte count, which may therefore underestimate the patient's immunological status [49].

In summary, severe malaria in HIV-coinfected children presents with more severe acidosis, anemia and respiratory distress, more complications, and comorbidity, causing higher mortality and prolonged hospitalization in survivors. HIV coinfection is associated with a higher estimated total parasite burden, which is strongly associated with the observed increased severity. Early recognition of HIV coinfection is important for several reasons. Higher case fatality and more frequent complications warrant more intense monitoring and a low threshold for additional investigations to diagnose concomitant invasive bacterial infections, including chest X-ray, blood culture, and lumbar puncture with CSF examination. Since concomitant pneumonia, sepsis, and severe anemia are common, prompt parenteral antimalarial and antibiotic treatment, and availability of supportive treatments (including oxygen therapy and blood transfusion) are of extra importance in this group.

Notes

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