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Cell-Free Hemoglobin Is Associated With Increased Vascular Resistance and Reduced Peripheral Perfusion in Severe Malaria

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Background. In severe falciparum malaria, unlike sepsis, hypotension on admission is uncommon. We hypothesized that low nitric oxide bioavailability due to the presence of cell-free hemoglobin (CFH) increases vascular tone in severe malaria.

Methods. Patients with severe malaria (n = 119), uncomplicated malaria (n = 91), or suspected bacterial sepsis (n = 56), as well as healthy participants (n = 50), were recruited. The systemic vascular resistance index (SVRI) was estimated from the echocardiographic cardiac index and the mean arterial pressure.

Results. SVRI and hematocrit levels were lower and plasma CFH and asymmetric dimethylarginine levels were higher in patients with malaria, compared with healthy participants. In multivariate linear regression models for mean arterial pressure or SVRI in patients with severe malaria, hematocrit and CFH but not asymmetric dimethylarginine were significant predictors. The SVRI was lower in patients with suspected bacterial sepsis than in those with severe malaria, after adjustment for hematocrit and age. Plasma CFH levels correlated positively with the core-peripheral temperature gradient and plasma lactate levels and inversely with the perfusion index. Impaired peripheral perfusion, as reflected by a low perfusion index or a high core-peripheral temperature gradient, predicted mortality in patients with severe malaria.

Conclusions. CFH is associated with mean arterial pressure, SVRI, and peripheral perfusion in patients with severe malaria. This may be mediated through the nitric oxide scavenging potency of CFH, increasing basal vascular tone and impairing tissue perfusion.

Keywords. Severe malaria; hemodynamics; vascular resistance; vascular tone; nitric oxide; cell-free hemoglobin.

Severe malaria is a multiorgan disease defined by the presence of 1 or more diverse syndromes, including coma, metabolic acidosis, hyperparasitemia, severe anemia, and renal failure. Of these, severity of coma, metabolic acidosis, and renal failure strongly predict a fatal course [1].

Despite the high number of circulating pathogens during malaria relative to bacteremic sepsis, hypotensive shock is less common on admission among patients with malaria than among those with sepsis, although differences in criteria make a direct comparison difficult. In large trials of treatment for severe malaria, the prevalence of decompensated shock on presentation was around 5% in children and 12% in adults [2, 3]. In contrast, shock was observed in 30%–85% of patients with sepsis in intensive care units [4–6]. The disparity in the prevalence of shock between patients with severe malaria and those with sepsis suggests fundamental differences in the pathophysiology.

Studies of adults with severe malaria have variably reported the cardiac index and systemic vascular resistance index (SVRI) to be low, normal, or increased [7–10]. In comparison, early bacterial sepsis is reported as typically being associated with a high cardiac index and a low SVRI [11]. The decrease in the SVRI during sepsis is mainly caused by vasodilation [12]. In severe sepsis, the proinflammatory immune response results in systemic vasodilation through a variety of pathways [11, 13–15]. In both malaria and sepsis, the ratio of the level of the nitric oxide (NO) precursor L-arginine to the level of the NO synthase inhibitor asymmetric dimethylarginine falls, limiting microvascular production of NO. In consequence, in both diseases, NO

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levels fall, and NO-dependent reactive hyperemia is impaired [16, 17]. In malaria, however, additional factors limit vascular NO bioavailability further. Intravascular hemolysis of infected and uninfected red blood cells causes a rise in cell-free hemoglobin (CFH), which scavenges NO and is associated with a reduction in reactive hyperemia [18]. The effects of a reduction in NO bioavailability by cell-free hemoglobin on macrovascular hemodynamics and resting vascular tone have not been established in severe malaria. Vascular resistance is determined by both the geometry of the vascular system and blood viscosity [19]. Blood viscosity is determined by hematocrit and, to a lesser extent, plasma total protein composition and concentration, red blood cell deformability, and red blood cell aggregation. The relative contributions of these factors depend on the sheer stress applied to this non-Newtonian fluid [20].

In this study, we assess potential determinants of mean arterial pressure (MAP), SVRI, and peripheral perfusion in severe malaria to examine the hypothesis that, in severe malaria, cell-free hemoglobin decreases NO bioavailability, leading to an increased SVRI and mean arterial pressure, reduced peripheral perfusion, and impaired tissue perfusion. For comparison, we assessed the same variables in patients with uncomplicated malaria, patients with suspected bacterial sepsis, and healthy participants.

METHODS

The study was approved by the Oxford Tropical Research Ethics Committee and the Chittagong Medical College Ethics Committee and ISPAT General Hospital ethics committee. Consecutive eligible inpatients with malaria were enrolled at Chittagong Medical College Hospital (Chittagong, Bangladesh) between 2011 and 2014 and ISPAT General Hospital (Rourkela, India) in 2011 during the malaria season. The inclusion criteria for malaria was presence of asexual falciparum parasitemia on blood film examination. The only exclusion criteria was absence of consent however for practical reasons only patients admitted to adult medical wards (which included children down to 10 years old) participated in hemodynamic studies. Severe malaria was defined as previously [21] as malaria with the presence of at least one of the following criteria: coma (Glasgow coma scale <11), parasite count of >100 000 parasites/μL with severe anemia (hematocrit <20%) or jaundice (bilirubin level of >2.5 mg/dL), renal impairment (serum creatinine level of >3 mg/dL and/or anuria), pulmonary edema, spontaneous bleeding, at least 2 generalized convulsions in 24 hours, acidosis (venous plasma bicarbonate level of <15 mmol/L), hyperparasitemia (peripheral asexual stage parasitemia level of >10%), hyperlactatemia (venous plasma lactate level of >4 mmol/L), hypoglycemia (blood glucose level of <40 mg/dL), shock (systolic blood pressure of <80 mmHg with cool extremities). Patients with suspected bacterial sepsis were recruited during the same timeframe and from the same sites as the malaria patients. The inclusion criteria used for suspected bacterial sepsis were suspected bacterial infection and at least 2 of the 4 systemic inflammatory response criteria [22]. Blood films were checked for suspected bacterial sepsis patients to confirm they did not have malaria. Healthy subjects with no known acute or chronic illness, including hypertension and diabetes mellitus were recruited locally. Informed, written consent was sought from participants or, in cases where they were unable to give consent or aged under 18, from a legally acceptable representative.

On enrolment, clinical history and examination were performed and a venous blood sample was taken. Blood pressure was measured noninvasively and MAP calculated as 1/3*systolic blood pressure + 2/3*diastolic blood pressure from a single measurement. Transthoracic echocardiography was used to estimate the cardiac index, based on the left ventricular outflow tract diameter, the aortic velocity time integral, heart rate and estimated body surface area [23, 24]. SVRI was calculated as the ratio of MAP to cardiac index. As a measure of peripheral vasoconstriction, in 2012–2014 the core-peripheral temperature gradient [25] was calculated as the temperature difference between the tympanic temperature (Braun Thermoscan, Kronberg, Germany) and dorsum of the foot (between the extensor tendons of the first and second toe, about 2–3 cm proximal to the base of the web space) using an infrared thermometer (Thermofocus Model 0800H4, Technimed, Varese, Italy). As an alternative index of peripheral perfusion, the perfusion index [26] was measured in 2012–2014 using a pulse oximeter (Radical-7, Masimo, Irvine, USA). The oximeter calculates the perfusion index as the ratio of the pulsatile to non-pulsatile signal, expressed as a percentage. Cell-free hemoglobin was measured by enzyme-linked immunosorbent assay (Bethyl, Montgomery, USA) in lithium heparin plasma. Plasma L-arginine and asymmetric dimethylarginine were measured by high performance liquid chromatography [27]. Red cell deformability was measured using a laser-assisted optical rotational red cell analyzer as previously described (LORCA) (Mechatronics, Hoorn, The Netherlands) [28]. Patients were followed up for recording of in hospital mortality.

Analysis

Correlations between variables were assessed using Spearman rank coefficient. Groups were compared using Kruskal-Wallis and Mann-Whitney *U* tests. χ^2 tests were used to assess association between categorical variables. Multiple linear regression was used to model the association between cardiac index, MAP or SVRI and possible determinants in severe malaria. Variables for which there was a P value of $< .05$ yielded by univariate correlation analysis were included the base model, with transformation where appropriate. Subsequently other possible determinants were added in turn and retained if they were significant at the $P < .05$ level. To assess if cell free hemoglobin

was independently associated with lactate variables previously found to be independently associated with lactate [21] (parasitemia, PfHRP2, red cell deformability and total bilirubin) were included in the model alongside cell free hemoglobin. In order to give an impression of the extent of organ involvement in the malaria and suspected bacterial sepsis groups, 1 point was assigned for each of the following criteria and summed to give total number of criteria met; Glasgow coma score < 11, hematocrit < 20, creatinine level > 3 mg/dl, pulse oximeter oxygen saturation <90% on room air, base excess <−8, lactate level > 4 mmol/l, and clinical jaundice.

RESULTS

Demographic details, enrollment clinical and laboratory data, and outcomes of enrolled patients are shown in Table 1. 11% (13/119) of the patients with malaria were aged under 18 (minimum age 10). The group with severe malaria more frequently had evidence of severe organ dysfunction than those with suspected bacterial sepsis, with 115/119 (97%) of severe malaria patients having evidence of severe organ dysfunction as defined in the methods while only 25/56 (45%) of the suspected bacterial sepsis group did ($P < .001$). Hyperlactatemia was more frequent in the severe malaria group (54/119, 45%) than the suspected bacterial sepsis group (6/50, 12%; $P < .001$), whereas hypotension was more frequent in the suspected bacterial sepsis group (5/56; 9%) than the severe malaria group (2/119, 2%; $P = .001$). Of the patients with severe malaria and sepsis respectively, 2/119 (2%) and 7/56 (13%) were receiving vasopressors and 2/119 (2%) and 3/56 (5%) were being mechanically ventilated around the time of assessment. Table 2 details hemodynamic and related data. Cardiac index data was available from 94/119 (79%) patients with severe malaria, 82/91 (90%) patients with uncomplicated malaria, 44/56 (79%) with suspected bacterial sepsis and 43/50 (86%) healthy persons due to either technical or logistical constraints. Cell-free hemoglobin was higher in patients with severe malaria compared to uncomplicated malaria ($P < .001$) and suspected bacterial sepsis ($P < .001$) (Table 2).

Cardiac Index

Cardiac index was increased in patients with severe malaria relative to healthy subjects ($P < .001$) but similar to patients with uncomplicated malaria ($P = .19$) and suspected bacterial sepsis ($P = .90$) (Table 2). In severe malaria, cardiac index was inversely correlated with MAP (rho = -0.31, $P = .003$, N = 94) and hematocrit (Rho = -0.41, $P < .001$, N = 94). No correlations were found between cardiac index and other determinants of viscosity (total protein, red cell deformability) or variables potentially related to vascular tone (log cell-free hemoglobin, log arginine, log asymmetric dimethylarginine, age) (all $P > .05$). In multiple regression models for cardiac index adjusting for hematocrit, sequential addition of the above markers of tone or viscosity yielded no additional significant predictors in patients with severe malaria (all $P > .05$).

Determinants of MAP and SVRI

MAP was similar in severe and uncomplicated malaria ($P = .24$) and severe malaria and suspected bacterial sepsis ($P = .55$), but lower in severe malaria than in healthy persons ($P < .001$) (Table 2). We found no evidence of a difference in SVRI between severe and uncomplicated malaria ($P = .36$) or severe malaria and suspected bacterial sepsis $P = .83$), but strong evidence of a lower SVRI in severe malaria compared to healthy participants ($P < .001$) (Table 2).

In severe malaria, MAP and SVRI were both correlated positively with hematocrit (rho = 0.44, $P < .001$, N = 117, and rho = 0.48, $P < .001$, N = 94 respectively). There were also positive correlations in severe malaria between plasma cell-free hemoglobin and MAP (rho = 0.19, $P = .04$, N = 112, Figure 1A) and between plasma cell-free hemoglobin and SVRI (rho = 0.25, $P = .017$, N = 92), Figure 1B). In addition, MAP correlated positively with plasma total protein (rho = 0.29, $P = .002$, N = 114) and age (rho = 0.28, $P = .002$, N = 117). Neither SVRI nor MAP correlated with red cell deformability, log asymmetric dimethylarginine, log arginine, or the arginine to asymmetric dimethylarginine ratio in severe malaria (all $P > .05$). There was no correlation between hematocrit and cell-free hemoglobin in severe malaria ($P > .05$).

In multivariate models for MAP and SVRI in patients with severe malaria using the significant univariate correlates identified, enrollment hematocrit, log plasma cell-free hemoglobin and log age (in the case of MAP) remained as the independent predictors (Table 3). Sequential addition of the other variables (log arginine, log asymmetric dimethylarginine, the arginine to asymmetric dimethylarginine ratio, red cell deformability) to the models for SVRI or MAP, yielded no additional significant predictors, nor did the addition of various interaction terms.

Figure 2 illustrates the relationship between hematocrit and SVRI in the different categories of patients. Notably, patients with suspected bacterial sepsis appear to have a lower SVRI for a given hematocrit suggesting they are more vasodilated. In order to compare vascular tone across the patient groups, multiple regression was performed adjusting for hematocrit (as a surrogate for viscosity) and log age. Participant category was included as a factor, with healthy participants comprising the reference group. Adjusting for hematocrit and log age, the SVRI was lower in suspected bacterial sepsis than severe malaria (F-test, $P = .002$) suggesting more prominent vasodilation in this group (Table 4).

Associations Between Measures of Vasoconstriction and Tissue Perfusion

The core-peripheral temperature gradient decreased from healthy participants to uncomplicated to severe malaria (test for trend $P = .001$) (Table 2). In severe malaria plasma

Table 1. Study Cohort Baseline Characteristics and Outcome, by Group

Variable	Healthy Control Group		Uncomplicated Malaria Group		Severe Malaria Group		Suspected Bacterial Sepsis Group		
	Value	Participants, No.	Value	Participants, No.	Value	Participants, No.	Value	Participants, No.	*P*[a]
Age, y	28 (24–35)	50	25 (20–40)	97	30 (22–40)	119	34 (25–51)	56	.23
Body surface area, m^2	1.68 (1.53–1.79)	50	1.51 (1.36–1.66)	97	1.53 (1.43–1.66)	119	1.49 (1.37–1.65)	55	<.001
Fever duration, d	Not applicable	…	7 (5–10)	97	7 (6–9)	118	9 (5–14)	53	.33
Intravenous fluid before enrollment, mL/kg[b]	Not applicable	…	19 (9–33)	50	20 (14–36)	70	15 (0–38)	24	<.001
Time from admission to enrollment, h	Not applicable	…	17 (10–21)	96	10 (2–17)	116	21 (10–42)	44	<.001
Chittagong enrollment site	50 (100)	50	81 (79)	103	110 (92)	119	51 (91)	56	<.001
Tympanic temperature, °C	36.7 (36.5–37)	50	37.6 (37–38.5)	97	38.2 (37.3–39)	117	38.4 (37.6–39.2)	56	<.001
GCS	Not done	…	15 (15–15)	97	9 (8–13)	119	15 (13–15)	55	<.001
Venous pH	7.37 (7.35–7.39)	34	7.44 (7.4–7.47)	97	7.39 (7.33–7.44)	119	7.46 (7.41–7.5)	47	<.001
Venous base excess level, mmol/L	1.5 (−1–2)	34	−2 (−4–0)	97	−8 (−12 to −4)	119	−1 (−6–3)	50	<.001
Venous lactate level, mmol/L	1.1 (0.9–1.5)	34	1.5 (1.2–2)	97	3.8 (2.4–6.3)	119	1.7 (1.2–2.5)	50	<.001
Creatinine level, mg/dL	0.9 (0.7–1)	34	1 (0.8–1.2)	96	1.4 (1–3.5)	118	1.1 (0.9–1.4)	46	<.001
BUN level, mg/dL	8 (5–9)	34	15 (9–25)	97	41 (24–73)	119	14 (8–23)	47	<.001
Total bilirubin level, mg/dL	Not done	…	1 (0.6–1.9)	95	2 (1–5.3)	117	0.4 (0.3–0.7)	44	<.001
Parasite count, parasites/μL	Not done	…	13 138 (1480–56 131)	96	75 109 (12 208–282 751)	119	Not done	…	
Log PfHRP2 level, ng/mL	Not done	…	6.3 (5.2–7)	90	7.9 (7.3–8.7)	112	Not done	…	
White blood cell count, $\times 10^3$ cells/μL	8.6 (6.7–9.5)	29	6.1 (4.4–8.7)	95	9.3 (6.8–13)	111	11 (8–18.3)	49	<.001
Male sex	38 (76)	50	68 (70)	97	80 (67)	119	30 (54)	56	.07
Smoker	10 (20)	49	34 (35)	96	43 (38)	113	18 (38)	48	.16
GCS <11	Not applicable	…	0 (0)	97	81 (68)	119	12 (22)	55	<.001
Bicarbonate level <15 mmol/L	Not applicable	…	0 (0)	97	35 (29)	119	5 (10)	50	<.001
Lactate level >4 mmol/L	Not applicable	…	0 (0)	97	54 (45)	119	6 (12)	50	.003
Hematocrit <21%	Not applicable	…	15 (15)	97	30 (25)	119	2 (4)	51	.003
SBP <80 mm Hg	Not applicable	…	0 (0)	97	2 (2)	119	5 (9)	56	.001
Died	Not applicable	…	0 (0)	97	42 (35)	119	14 (25)	56	<.001

Data are median values (interquartile ranges) or no. (%) of participants, unless otherwise indicated.

Abbreviations: BUN, blood urea nitrogen; GCS, Glasgow coma score; PfHRP2, *Plasmodium falciparum* histidine rich protein; SBP, systolic blood pressure.

[a]By the Kruskal-Wallis rank test (for continuous data) or the χ^2 test (for binary data) across all 4 groups.

[b]These data were not available for all patients; lack of data does not mean they received no fluids.

Table 2. Baseline Hemodynamic Variables and Associated Variables, by Group

Variable	HC		UM		SM		SBS		*P*[a]			
	Value	Participants, No.	Value	Participants, No.	Value	Participants, No.	Value	Participants, No.	Overall	HC vs SM	UM vs SM	SBS vs SM
Hematocrit, %	43 (37–45)	49	30 (23–35)	97	26 (20–32)	119	35 (30–40)	52	<.001	<.001	.01	<.001
EI, by sheer stress												
At 1.7 Pa	0.21 (.18–.24)	25	0.21 (.17–.22)	69	0.18 (.14–.2)	98	0.22 (.19–.24)	32	<.001	<.001	<.001	<.001
At 5.3 Pa	0.41 (.37–.43)	25	0.37 (.34–.39)	69	0.35 (.28–.37)	98	0.39 (.34–.41)	32	<.001	<.001	<.001	<.001
Total protein level, g/dL	Not done	…	7 (5–7)	11	5 (5–6)	116	6 (6–7)	44	<.001		.018	<.001
Arginine level, μmol/L	90 (77–107)	47	51 (38–62)	90	58 (45–70)	109	47 (39–63)	39	<.001	<.001	.002	.006
ADMA level, μmol/L	0.53 (.47–.57)	47	0.62 (.52–.76)	90	0.72 (.54–.93)	109	0.5 (.41–.68)	39	<.001	<.001	.004	<.001
Ratio of arginine to ADMA levels	184 (149–213)	47	82 (62–105)	90	76 (65–97)	109	91 (68–126)	39	<.001	<.001	.676	.035
Cell-free hemoglobin level, μmol/L	3.3 (1.7–9.1)	22	3.7 (1.8–5.2)	87	7.4 (3.7–14.7)	114	2.4 (.7–5.6)	35	<.001	.009	<.001	<.001
Heart rate, beats/min	76.5 (69–82)	50	100 (88–113)	97	113 (95–132)	119	110 (99–125)	56	<.001	<.001	<.001	.603
Cardiac index[b]	2.7 (2.3–3.1)	43	3.8 (3.1–4.4)	82	4 (3.3–4.8)	94	4 (3.1–4.8)	44	<.001	<.001	.188	.895
MAP, mm Hg	93 (84–99)	50	80 (72–87)	97	82 (73–91)	117	85 (71–95)	55	<.001	<.001	.235	.55
SVRI[c]	2789 (2457–3293)	43	1662 (1385–2333)	82	1658 (1291–2094)	94	1637 (1297–2141)	44	<.001	<.001	.364	.83
Perfusion index	8.65 (5.9–11)	34	4.75 (2.5–7.7)	46	4.6 (2–8.2)	55	3.7 (1.75–7.65)	24	<.001	<.001	.978	.409
CPTG, °C[d]	3.3 (2.5–4)	34	2.6 (1.8–3.5)	41	1.9 (1.3–3.2)	64	3.4 (1.8–4.8)	11	.005	.001	.054	.072

Data are median values (interquartile ranges), unless otherwise indicated.

Abbreviations: ADMA, asymmetric dimethylarginine; EI, red blood cell elongation index (a measure of red blood cell deformability); HC, healthy control group; JVP, jugular venous pressure; MAP, mean arterial pressure; SBS, suspected bacterial sepsis group; SM, severe malaria group; UM, uncomplicated malaria group.

[a]By the Kruskal-Wallis rank test or Mann-Whitney *U* test.

[b]Calculated as L/min/m^2.

[c]The systemic vascular resistance index (SVRI) was calculated as dynes.s/cm^5/m^2.

[d]The core-peripheral temperature gradient (CPTG) was calculated as the temperature difference between the tympanic temperature and the dorsum of the foot.

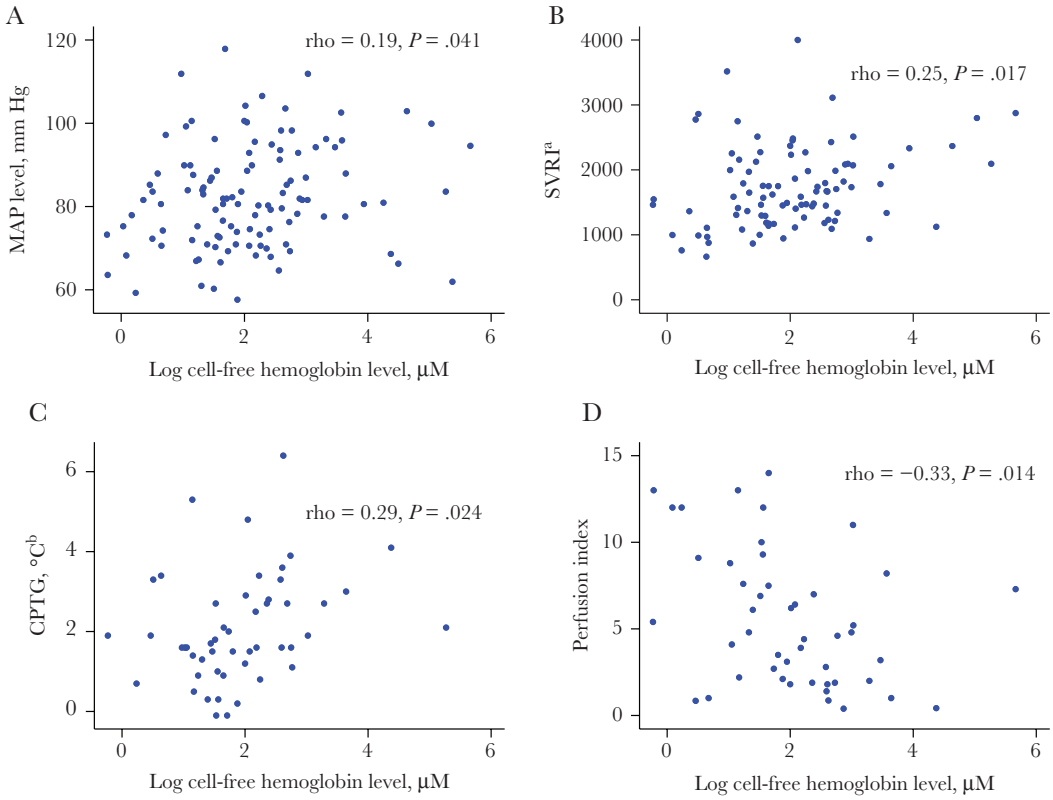

Figure 1. Relationships between cell-free hemoglobin levels and indices of vascular tone. [a]The systemic vascular resistance index (SVRI) was calculated as dynes.s/cm^5/m^2. [b]The core-peripheral temperature gradient (CPTG) was calculated as the temperature difference between the tympanic temperature and the dorsum of the foot.

cell-free hemoglobin was correlated positively with the core-peripheral temperature gradient and plasma lactate and negatively with the perfusion index (Table 5, Figure 1C and D), but not the core temperature (P = .08). There were no significant correlations between core-peripheral temperature gradient and plasma asymmetric dimethylarginine (P = .65) or L-arginine (P = .58) or hematocrit (P = .33) in severe malaria. In a multivariate model for plasma lactate in severe malaria, using the previously established predictors namely, plasma PfHRP2 concentration, parasite count, total bilirubin and red cell deformability, log cell-free hemoglobin (P = .014), red cell deformability at 1.7Pa (P = .028) and log parasite count (P = .001) remained significant predictors, whereas log PfHRP2 (P = .861) and total bilirubin (P = .432) did not contribute to the model (Supplementary Table 1).

Impaired peripheral perfusion was associated with mortality in severe malaria. A large core-peripheral temperature gradient (AUROC = 0.70 95%CI .56–.84) or a small perfusion index (AUROC = 0.30 95%CI .14–.45), reflecting reduced peripheral perfusion predicted death. As shown previously, lactate was associated with mortality (AUROC = 0.77 95%CI .68–.86). Core or peripheral temperature alone or cell free hemoglobin were not associated with mortality (P > .05).

Table 3. Linear Regression Coefficients for the Determinants of Mean Arterial Blood Pressure (MAP) and Systemic Vascular Resistance Index (SVRI) for Patients With Severe Malaria

	MAP, mm Hg (n = 110)		SVRI[a] (n = 92)	
Variable	β (95% CI)	*P*	β (95% CI)	*P*
Hematocrit, %	0.56 (.27–.85)	<.001	39.54 (24.23–54.85)	<.001
Log cell-free hemoglobin level, µmol/L	1.87 (.19–3.54)	.029	100.73 (.32–201.14)	.049
Log age, y	7.42 (2.51–12.33)	.003	Not applicable	
Total protein level, g/dL	2.18 (−1.21–5.57)	.205	Not applicable	

R^2 was calculated as 0.3 for the MAP model and 0.28 for the SVRI model.

Abbreviation: CI, confidence interval.

[a]Calculated as dynes.s/cm^5/m^2.

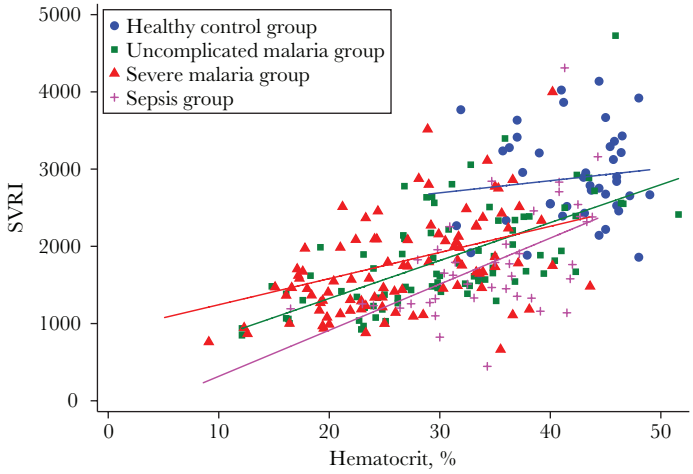

Figure 2. Relationship between the systemic vascular resistance index (SVRI; calculated as dynes.s/cm^5/m^2) and hematocrit in the different patient groups. Linear regression was used to model the relationship between hematocrit and SVRI within the different patient groups. Regression lines represent the predicted relationship. Data from 1 patient with severe malaria with an estimated SVRI of 8444 are not shown.

DISCUSSION

Whilst hypotension on enrolment was more frequent in the suspected bacterial sepsis group than the severe malaria group, the SVRI was similarly low. The low SVRI seen in severe malaria appears to have a greater contribution from anemia reducing blood viscosity and a lesser contribution from vasodilation than suspected bacterial sepsis. Cell-free hemoglobin was elevated in severe malaria and positively associated with SVRI and decreased peripheral perfusion (core-peripheral temperature gradient and perfusion index) consistent with cell-free hemoglobin mediated NO scavenging limiting the vasodilation seen in severe malaria and reducing the occurrence of hypotension. Cell-free hemoglobin was also associated with elevated plasma lactate concentrations, consistent with a role in impairing microvascular perfusion. Reduced peripheral perfusion predicted mortality in severe malaria.

SVRI is determined both by blood viscosity and vascular geometry [19]. Hematocrit is the main determinant of whole blood viscosity and consistent with this hematocrit and SVRI were strongly positively correlated in severe malaria. After adjusting for hematocrit, the SVRI was still lower in patients with malaria than in healthy participants (Table 4), implying that vasodilation in addition to a reduction in blood viscosity contributes to the fall In SVRI in severe malaria. Compared to patients with malaria, patients with suspected bacterial sepsis had a lower SVRI after adjustment for hematocrit, consistent with severe malaria patients being less vasodilated than septic patients. This is more notable given the suspected bacterial sepsis group was generally less severely unwell than the severe malaria group. Cell-free hemoglobin concentrations were increased in severe malaria relative to suspected bacterial sepsis and healthy persons, and were positively correlated with SVRI and MAP. Scavenging of the vasodilator NO by cell-free hemoglobin may serve to increase MAP and SVRI, reducing the frequency of hypotension in severe malaria relative to sepsis. Supporting the concept of cell-free hemoglobin mediated vasoconstriction, cell-free hemoglobin correlated with the core-peripheral temperature gradient and the perfusion index as

Table 4. Linear Regression Coefficients for the Determinants of Mean Arterial Blood Pressure (MAP) and Systemic Vascular Resistance Index (SVRI) Across All Patient Groups

	MAP, mm Hg (n = 314)		SVRI[a] (n = 258)	
Variable	β (95% CI)	*P*	β (95% CI)	*P*
Hematocrit, %	0.63 (.5 to –.77)	.001	43.8 (30.87 to –56.73)	.002
Age, y	5.41 (.58 to –10.25)	.038	337.21 (6.65 to –667.76)	.048
Uncomplicated malaria	–3.9 (–5.51 to –2.29)	.005	–542.77 (–691.68 to –393.86)	.001
Severe malaria	–0.08 (–2.06 to –1.89)	.902	–486.46 (–684.79 to –288.13)	.004
Sepsis	–4.39 (–5.63 to –3.16)	.001	–847.49 (–970.33 to –724.65)	<.001

Groups (uncomplicated malaria, severe malaria, and sepsis) were introduced as independent binary variables for this analysis. One outlier with severe malaria and an estimated SVRI of 8444 was excluded. R^2 was calculated as 0.25 for the MAP model and 0.5 for the SVRI model.

Abbreviation: CI, confidence interval.

[a]Calculated as dynes.s/cm^5/m^2.

Table 5. Relationship Between Indices of Vascular Tone, Parasite Burden, and Organ Function in Severe Malaria

Variable	SVRI			MAP			CPTG[a]			Perfusion Index			CFH Level			Parasitemia Level			PfHRP2			Lactate Level		
	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]	ρ	*P*	No.[a]
MAP	0.7	<.0001	93	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
CPTG[b]	0.16	.25	52	0.11	.41	63	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
Perfusion index	−0.06	.68	47	−0.25	.07	55	−0.39	.01	42	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
CFH level	0.25	.02	92	0.19	.04	112	0.29	.02	62	−0.33	.01	55	…	…	…	…	…	…	…	…	…	…	…	…
Parasitemia level	−0.2	.06	94	−0.11	.23	117	−0.04	.73	64	−0.09	.51	55	0.05	.62	114	…	…	…	…	…	…	…	…	…
PfHRP2	0.24	.03	88	0.06	.57	110	0.24	.07	57	−0.21	.14	52	0.53	<.0001	108	0.17	.07	112	…	…	…	…	…	…
Lactate level	−0.12	.25	94	0.05	.56	117	0.25	.049	64	−0.47	.003	55	0.21	.02	114	0.43	<.0001	119	0.28	.003	112	…	…	…
GCS	−0.19	.06	94	−0.07	.46	117	−0.16	.2	64	0.2	.14	55	−0.23	.01	114	0.25	.01	119	−0.07	.48	112	−0.16	.08	119

Relationship between markers of vasoconstriction, schizogony, and organ dysfunction.

Abbreviations: CFH, cell-free hemoglobin; CPTG, core-peripheral temperature gradient; GCS, Glasgow coma score; MAP, mean arterial pressure; PfHRP2, *Plasmodium falciparum* histidine rich protein 2; SVRI, systemic vascular resistance index.

[a]Data are no. of participants.

[b]The core-peripheral temperature gradient (CPTG) was calculated as the temperature difference between the tympanic temperature and the dorsum of the foot.

markers of peripheral vascular tone. In malaria, a reduction in SVRI due predominantly to low blood viscosity as opposed to vasodilation may result in less hypotension, as the reduction in blood viscosity may reduce the resistance to venous return increasing the preload, while reducing the afterload [29–31], maintaining the stroke index and cardiac output. Although NO scavenging may help preserve perfusion pressure in severe malaria, it may hinder microcirculatory flow and tissue perfusion, as cell-free hemoglobin concentrations and peripheral perfusion indices were correlated with an increase in plasma lactate as a crude measure of reduced tissue perfusion.

Previous studies in healthy individuals and other diseases have found similar effects of cell-free hemoglobin scavenging on resting vascular tone, including an increase in SVRI following transfusion of cell-free hemoglobin substitutes [32]. The impact of cell-free hemoglobin on dynamic but not resting state vascular tone has also been described previously in malaria: cell-free hemoglobin is inversely correlated with reactive hyperemia [18]. The association between cell-free hemoglobin and vasoconstriction could also be explained by the vasoconstricting effects of lipid peroxidation products generated by oxidized cell-free hemoglobin [33]. However, studies in dogs have suggested however that plasma oxyhemoglobin, as opposed to methemoglobin, is mediating vasoconstriction, consistent with NO scavenging-mediated as opposed to lipid peroxidation-mediated vasoconstriction [34].

Further support for the hypothesis that cell-free hemoglobin impairs microvascular perfusion through NO scavenging comes from the correlation between cell-free hemoglobin and plasma lactate in severe malaria, independent of PfHRP2 [18, 35]. Previous studies have shown that hyperlactatemia in severe malaria is multifactorial, due to a combination of impaired microcirculatory flow related to sequestration [36], impaired red cell deformability [28], clumping of infected erythrocytes (rosetting and autoagglutination) [37, 38] and impaired hepatic lactate clearance [39]. Consistent with this multifactorial origin of hyperlactatemia, parasite count and red cell deformability remained alongside cell-free hemoglobin as predictors of the plasma lactate concentration in our multivariate model. The strong inverse relationship we found between perfusion index and lactate was notable and may be of clinical utility. In severe sepsis perfusion index has been proposed as a marker of the adequacy of resuscitation [25, 40].

In addition to NO scavenging by cell-free hemoglobin, NO bioavailability is also reduced in severe malaria by depletion of its precursor, L-arginine, and increased levels of the NO synthase inhibitor, asymmetric dimethylarginine [41, 42]. In severe malaria we found no association between plasma arginine, asymmetric dimethylarginine, or the arginine/asymmetric dimethylarginine ratio and indices affected by vascular tone: MAP, SVRI, or the core-peripheral temperature gradient. Previous studies in severe malaria have found longitudinal

but not baseline associations between plasma L-arginine concentrations and reactive hyperemia indices [42, 43]. Plasma asymmetric dimethylarginine has been previously correlated with baseline exhaled NO in severe malaria, and with reactive hyperemia, however the latter only in adjusted analyses [41]. It is possible that the lack of correlation between baseline arginine and asymmetric dimethylarginine and SVRI or MAP observed here, and with reactive hyperemia in unadjusted analysis previously [42], could reflect residual confounding by unmeasured variables. These could include factors that limit NO synthesis besides the relative low bioavailability of the substrate. Recent evidence has indicated that availability of the cofactor of NO synthase, tetrahydrobiopterin is limited in severe malaria [44, 45]. Additionally, since NO synthase (NOS) is an intracellular enzyme, plasma L-arginine concentrations may not correlate well with the intracellular concentrations available to the enzyme, and this may vary between patients. Nevertheless, consistent with low L-arginine bioavailability limiting NO production, infusion of L-arginine in moderately severe malaria increases both exhaled NO and reactive hyperemia [42].

The study has some limitations. This study used noninvasive hemodynamic measurements to maximize acceptability for participants. Whole blood and plasma viscosity was not directly measured, and data on other vasoactive compounds were not available. NO was not measured directly due to difficulties in quantifying this unstable molecule. This study comprised mostly adults; adolescents were also included as there is no reason to suspect a different relationship between SVRI and peripheral perfusion with cell free hemoglobin than seen in adults. Since SVRI is indexed to BSA, it is adjusted for body size. Unlike for children less than 10, the frequency of the different malaria severity features seen in adolescents approximates that seen in adults [46]. Owing to the lack of diagnostic capacity it was not possible to confirm a bacterial etiology in the suspected bacterial sepsis group. The suspected bacterial sepsis group contained patients with both severe and "uncomplicated" suspected bacterial sepsis, with only the minority being hypotensive or having a high lactate. This study was started before the Sepsis-3 criteria were published in 2016; hence, we used systemic inflammatory response criteria for recruitment [47].

This study provides evidence for the role of NO scavenging by cell-free hemoglobin in the regulation of basal vascular tone in severe malaria, and associates the resultant reduced peripheral perfusion with elevated plasma lactate. Reduced peripheral perfusion was associated with mortality in severe malaria. This supports the adjunctive therapy approaches to increase NO bioavailability at the microcirculatory level to improve tissue perfusion in severe malaria. However, since refractory shock can develop late in the disease and is common prior to death [48], vasodilatory therapy should be provided with caution. The core-peripheral temperature gradient and perfusion index may be useful pharmacodynamic measures for assessing NO bioavailability.

Supplementary Data

Supplementary materials are available at *The Journal of 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

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