Direct In Vivo Assessment of Microcirculatory Dysfunction in Severe Falciparum Malaria

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Background. This study sought to describe and quantify microcirculatory changes in the mucosal surfaces of patients with severe malaria, by direct in vivo observation using orthogonal polarization spectral (OPS) imaging.

Methods. The microcirculation in the rectal mucosa of adult patients with severe malaria was assessed by use of OPS imaging, at admission and then daily. Comparison groups comprised patients with uncomplicated falciparum malaria, patients with bacterial sepsis, and healthy individuals.

Results. Erythrocyte velocities were measured directly in 43 adult patients with severe falciparum malaria, of whom 20 died. Microcirculatory blood flow was markedly disturbed, with heterogeneous obstruction that was proportional to severity of disease. Blocked capillaries were found in 29 patients (67%) and were associated with concurrent hyperdynamic blood flow (erythrocyte velocity, >750 mm/s) in adjacent vessels in 27 patients (93%). The proportion of blocked capillaries correlated with the base deficit in plasma and with the concentration of lactate. Abnormalities disappeared when the patients recovered. In healthy individuals and in patients with uncomplicated malaria or sepsis, no stagnant erythrocytes were detected, and, in patients with sepsis, hyperdynamic blood flow was prominent.

Conclusion. Patients with severe falciparum malaria show extensive microvascular obstruction that is proportional to the severity of the disease. This finding underscores the prominent role that microvascular obstruction plays in the pathophysiology of severe malaria and illustrates the fundamental difference between the microvascular pathophysiology of malaria and that of bacterial sepsis.
this results in clear intravital images of RBCs flowing in the microcirculation. We have used this technique to study microcirculatory changes in patients with severe falciparum malaria.

**PATIENTS AND METHODS**

*Patients.* Adult patients who were consecutively admitted to the Chittagong Medical College Hospital (Chittagong, Bangladesh) with severe falciparum malaria were included, provided that written informed consent had been obtained from the patient or attending relative. Patients with severe malaria were enrolled as part of a randomized trial evaluating the antioxidant N-acetylcysteine as adjunctive treatment in severe malaria, the results of which will be reported separately. Ethical approval for this study was obtained from the Bangladesh Medical Research Council and the Oxford Tropical Medicine Research Ethical Committee. Severity of the disease was classified according to standard criteria [9]. For all of the patients, a detailed medical history was obtained, and a physical examination performed. Antimalarial treatment consisted of either parenteral quinine (*n* = 9) or artesunate (*n* = 34). Supportive care was given, as described previously [1], but the availability of mechanical ventilation and of renal replacement therapy was limited. Blood pressure was measured by conventional sphygmomanometry. Oxygen saturation in blood was measured noninvasively by pulse oxymetry (Nonin Medical). Patients with uncomplicated falciparum malaria and sepsis were recruited from Mae Sot Hospital (Tak Province, Thailand) and were assessed at admission only. Uncomplicated falciparum malaria was defined as slide-proven malaria that does not fulfill the criteria for severity [9], and sepsis was defined as the presence of an infection in combination with systemic inflammatory-response syndrome, as indicated by at least 3 of the following criteria: (1) a core temperature of ≥38°C or ≤36°C; (2) a heartbeat of ≥90 beats/min; (3) either a respiratory rate of ≥20 breaths/min, a PaCO₂ of ≥32 mmHg, or the use of mechanical ventilation for an acute respiratory process; and (4) either a white-cell count of ≥12 × 10⁹/L or ≤4 × 10⁹/L or a differential count showing >10% immature neutrophils [10].

*Laboratory methods.* At admission, thick and thin films were taken from peripheral-blood samples and were stained with Field’s stain for parasite count. Venous-blood samples were taken at admission, at 6 h after admission, and then every 12 h for parasite count, hematocrit, routine biochemistry, acid-base parameters, and concentration of lactate. The latter 2 were assessed by use of an i-STAT handheld biochemical analyzer (i-STAT).

*OPS imaging.* An OPS device (either Cytoscan, from Cytometrics, or Microscan, from Microvision Medical) was used for the assessment of microcirculatory blood flow. Initial pilot studies assessing the blood flow in the buccal and rectal mucosae indicated that quantitation would be more reliable in the rectal mucosa, because of its regular vascular geometry. Care was taken that the blood flow was not impaired by the pressure of the thin probe on the mucosa. For quantitative analysis, we used video images that were stable for ≥15 s, and assessments were made on 3 different fields. The video recordings were imported into Final Cut Pro (version 3.0.2; Apple), and were analyzed by use of the OpenLab 3.1.5 image-analysis software package (Improvision). The vessels in the rectal mucosa form a hexagonal pattern of capillaries that surround the crypts at the center. In all of the vessels of the hexagon, the blood flow was assessed by tracing the movement of individual erythrocytes over time, by the use of video recordings, and measuring their speed. Only individual erythrocytes that could be tracked over 3 successive frames were assessed. Because the frame rate of the camera was 30 Hz, and because the length of the vessel bordering the crypts was ≤50 µm, only erythrocytes with speeds ≤750 µm/s could be assessed quantitatively; speeds >750 µm/s were denoted as “hyperdynamic.” Empty capillaries were not assessed. A mean of 50 (95% CI, 37–62) vessels was assessed for each field, and 3 different fields were assessed for each patient.

*Statistical analysis.* Statistical analysis was performed with SPSS statistical software (version 11.0; SPSS). Intergroup differences in normally distributed continuous variables and variables log-transformed toward normality were compared by use of the unpaired Student’s t test, and correlation coefficients for pairs of variables were determined by Pearson’s method, for normally distributed variables, and by Spearman’s method, for the remainder. For nonnormally distributed variables, the differences between the groups were compared by use of the Kruskal–Wallis test. A stepwise forward logistic-regression model was constructed to evaluate the parameters associated with capillary flow and concentrations of lactate in plasma.

**RESULTS**

Of the 53 consecutive patients with severe malaria who were admitted to prospective clinical studies at Chittagong Medical College Hospital, there were 43 in whom the microcirculation of the rectal mucosa could be assessed satisfactorily. Consent for this investigation was not obtained from 6 patients or their relatives, and, in another 4 patients, fecal contamination prevented us from adequately viewing the small blood vessels. Baseline characteristics are shown in table 1. Of the 43 patients with severe malaria, 36 (84%) had cerebral malaria (Glasgow Coma Scale, <11), 35 (81%) were acidic (standard base excess, <3.3 mmol/L), 7 (16%) had renal failure (serum creatinine, >3.0 mg/dL), 4 had severe anemia (hematocrit, <20%), whereas 5 had hyperparasitemia (>10% of RBCs), and 32 (74%) had jaundice (total bilirubin >2.5 mg/dL). Only 1 patient (2%) was in shock at admission, with a systolic blood pressure of 60 mm Hg. This patient quickly recovered after fluid resuscitation. Patients were
treated with either intravenous quinine (n = 9) or artesunate (n = 34); 20 patients (47%) died. In the preliminary pilot study, the microcirculation in sublingual mucosa was viewed in another 20 patients. Bruxism, a common feature of cerebral malaria, frequently prevented us from introducing the probe orally, and, because the vasculature was irregular in caliper, this route was not pursued further. The findings were obvious and striking: heterogeneous obstruction of microcirculatory blood flow during the acute phase of severe falciparum malaria. The microcirculatory obstruction, in terms of the proportion of vessels involved and the degree of obstruction, was proportional to the severity of the disease, and it decreased on clinical recovery. Vessels with little or no blood flow were often seen adjacent to vessels with hyperdynamic blood flow. The changes were more prominent in the rectal mucosa than in the sublingual mucosa (see figure 1; this figure is a still of the video, which is available in the online edition of the Journal).

A quantitative assessment of the erythrocyte velocities in the rectal mucosa confirmed the visual impression that they differed even within individual patients. Blocked capillaries were found in 29 (67%) of the 43 patients, 27 (93%) of whom also showed hyperdynamic blood flow (erythrocyte velocity, >750 μm/s). Overall, in the 43 patients with severe malaria, the median proportion of blocked capillaries was 8% (range, 0%–63%), whereas the median proportion of capillaries with hyperdynamic blood flow was 24% (range, 0%–77%), compared with 14% (range, 6%–21%) in healthy individuals. Video recordings of the rectal mucosa in patients with sepsis showed prominent hyperdynamic blood flow, with no evidence of blocked capillaries (see figures 1 and 2; figure 1 is a still of the video, which is available in the online edition of the Journal). The median (SD) proportion of capillaries showing erythrocyte velocities >750 μm/s was 71% (range, 28%–100%). In the 6 patients with sepsis, the source of the sepsis (no. of cases) was endometritis (1), necrotizing fasciitis (1), urosepsis (2), and pneumonia (2); the mean concentration of lactate in plasma was 3.5 mmol/L (95% CI, 2.5–4.4 mmol/L).

### Table 1. Baseline characteristics of patients with severe falciparum malaria, stratified in terms of disease outcome.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Survivors (n = 23)</th>
<th>Fatal cases (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>32 (16–65)</td>
<td>36 (16–60)</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>17/6</td>
<td>15/5</td>
</tr>
<tr>
<td>Blood pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>108 (100–116)</td>
<td>109 (101–117)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>68 (63–73)</td>
<td>66 (61–72)</td>
</tr>
<tr>
<td>Pulse rate, beats per min</td>
<td>109 (102–116)</td>
<td>114 (104–125)</td>
</tr>
<tr>
<td>O₂ saturation on air, %</td>
<td>95 (94–96)</td>
<td>94 (92–97)</td>
</tr>
<tr>
<td>Glasgow Coma Scale, median (interquartile range) (of 15)</td>
<td>8 (6–11)</td>
<td>6 (4–9)</td>
</tr>
<tr>
<td>Parasitemia, geometric mean (95% CI), organisms/μL</td>
<td>33,350 (14,760–75,352)</td>
<td>27,694 (8367–91,685)</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>10.7 (9.5–11.9)</td>
<td>9.5 (8.3–10.6)</td>
</tr>
<tr>
<td>Plasma creatinine, mg/dL</td>
<td>1.8 (1.4–2.2)</td>
<td>2.1 (1.3–3.0)</td>
</tr>
<tr>
<td>Total plasma bilirubin, mg/dL</td>
<td>8.6 (4.6–12.5)</td>
<td>10.0 (5.1–14.9)</td>
</tr>
<tr>
<td>Standard base deficit, mmol/L</td>
<td>7.1 (4.6–9.5)</td>
<td>13.3 (9.6–17.1)</td>
</tr>
<tr>
<td>Plasma lactate, mmol/L</td>
<td>5.3 (3.9–6.7)</td>
<td>7.8 (5.3–10.3)</td>
</tr>
</tbody>
</table>

**NOTE.** Data mean (95% confidence interval [CI]) values, unless otherwise indicated.
mmol/L); 2 patients died. All these patients were volume resuscitated, 4 patients received dopamine; the median dose was 10 μg/kg/min (range, 4–12 μg/kg/min). None of the patients with sepsis were in shock at the time of OPS imaging. Their median systolic blood pressure was 101 mmHg (range, 88–140 mmHg), and their median diastolic blood pressure was 51 mmHg (range, 48–70 mmHg) (all with warm peripheries). Their median pulse rate was 122/min (range, 108–144/min), their median temperature was 38.9°C (range, 38.5°C–40.2°C), their median respiratory rate was 28/min (range, 20–36/min), and the peripheral-blood leukocyte count was 13.5 × 10⁹/L. None of these 6 patients received mechanical ventilation at the time of assessment.

Both the proportion of blocked capillaries and the proportion of capillaries with hyperdynamic blood flow were significantly different between the groups (P = .001 and P = .017, respectively). In the patients with severe malaria, the fraction of blocked capillaries correlated positively both with the base deficit in plasma (r = 0.66 and P = .001) and with the concentration of lactate in venous plasma (r = 0.54 and P = .001) (figure 3). This association remained significant in a stepwise linear regression model, with the base deficit or the concentration of lactate in plasma as the dependent variable and with the percentage of blocked capillaries, peripheral-blood parasitemia, blood pressure, and oxygen saturation (assessed by pulse oximetry) as covariates (P = .014 and P = .03, respectively). Parasitemia also contributed to both models (for base deficit, standardized β = 0.298, t = 2.13, and P = .04; for lactate, standardized β = 0.297, t = 2.22, and P = .032). There was a weak but significant correlation between the proportion of blocked capillaries and the Glasgow Coma Scale (as a measure of coma depth): r = -0.32 and P = .04. The median fraction of blocked capillaries was 5% (range, 0%–30%) in patients who survived, compared with 12% (range, 0%–38%) in patients who died, although this difference did not reach statistical significance (P = .19).

Although the peripheral-blood parasitemia reflects parasitized erythrocytes that have not yet sequestered [11], it correlated positively with the fraction of obstructed capillaries (r = 0.41 and P = .007). The degree of microvascular obstruction was not associated with blood pressure or core temperature.

In patients who survived, the fraction of blocked capillaries declined sharply over time (figure 4). After 24 h, only 6 (26%) of 23 patients who survived still had some evidence of capillary obstruction, compared with 6 (67%) of 9 patients who died (P = .049). After 24 h, there was no correlation between a decrease in parasitemia and the presence of blocked capillaries. The erythrocyte velocities of the nonstagnant erythrocytes did not change significantly during the 2 days after admission.

DISCUSSION

The present study provides, for the first time, results of direct visualization and quantitation of microvascular sequestration in severe falciparum malaria. Blockage of capillary flow by stagnant erythrocytes was noticeable in the majority of patients. When the
patient recovered, microcirculatory blood flow was restored. These graphic, direct, in vivo observations confirm the evidence derived from pathological studies of fatal cases; in patients with severe falciparum malaria, the microcirculation is variably blocked by stagnant, presumably cytoadherent, erythrocytes [12–14]. The heterogeneity in microcirculatory blood flow is remarkable. Lethal disease presumably results when a critical threshold of obstruction in vital organs is exceeded.

These observations of a unique microvascular pathology in severe malaria are strikingly different from the blood-flow patterns observed in patients with bacterial sepsis. This difference emphasizes the fact that, although there are some similarities in clinical presentations, severe malaria and sepsis differ in their microvascular pathology. Hemodynamic shock, which is common in bacterial septicemia, occurs in only ~8% of adult patients with severe malaria [15]. When the same technique as has been described above is used, video recordings of the microcirculatory blood flow in the sublingual mucosa of patients with sepsis have shown a decrease in capillary density [16–18]. Heterogeneity in capillary perfusion and, in general, high microvascular flow were reported, but capillary blockage was absent. The present study describes, for the first time, hyperdynamic blood flow in the microcirculation of rectal mucosa of patients with sepsis. Because the focus of the present study was on severe malaria, only a limited number of patients with sepsis were studied, and none were in shock at the time of OPS imaging. A more extended study, in a wider variety of patients with sepsis, is under way. In severe malaria, the importance of tissue hypoxia is reflected in the consistent observation that both the elevated concentrations of lactate in plasma at admission, with an increased lactate:pyruvate ratio, and the severity of acidosis have a strong prognostic value for mortality in both adult and pediatric patients [15, 19, 20]. In bacterial septicemia, the concentration of lactate in plasma also has a prognostic significance for disease outcome, but, as in the present study, the lactate:pyruvate ratios are much lower, reflecting hypermetabolism rather than hypoxia as the cause and reflecting a pathogenesis that is probably more complex than that of simple ischemia [21]. Hyperdynamic blood flow in patients with sepsis cannot explain tissue hypoxia, because, even at high erythrocyte velocities, the unloading of oxygen is not compromised [22]. In patients with uncomplicated falciparum malaria, no microcirculatory obstruction could be observed. This finding is in accordance with the much lower sequestered parasite biomass in this group of patients with malaria [11]. The erythrocyte velocities in patients with uncomplicated falciparum malaria tended to be slightly higher than those in healthy individuals, presumably because of the cardiac-output increase associated with fever and mild anemia [23].

OPS imaging cannot distinguish between parasitized and noninfected RBCs as the cause of obstruction. However, its findings are consistent with those of postmortem studies of cerebral malaria, showing a heterogeneous distribution of capillaries packed and clogged with sequestered parasitized RBCs—with adjacent, apparently patent vessels [12, 13]. Autopsy studies have shown that sequestration is intense in the gut [14], a finding that is compatible with our in vivo observations. The extent of blood-flow obstruction in rectal mucosa correlated with the severity of metabolic acidosis and with concentrations of lactate in venous plasma. Lactic acidosis in severe malaria is thought to

Figure 3. Correlation between the fraction of obstructed capillaries in rectal mucosa and the concentration of lactate in plasma (upper panel) \( n = 43; \ r = 0.54 \) and \( P = .001 \) and correlation between the fraction of obstructed capillaries in rectal mucosa and the base deficit (lower panel) \( r = 0.66 \) and \( P = .001 \), at admission, in patients with severe falciparum malaria \( n = 43 \) \( r = 0.65 \) and \( P = .001 \). Closed circles denote fatal cases.

Figure 4. Fraction of capillaries with stagnant erythrocytes in rectal mucosa over time, after the start of antimalarial treatment in patients with severe falciparum malaria. Red dots denote fatal cases of the disease.
result mainly from tissue dysoxia, but decreased hepatic clearance also plays a role [15]. The results of the present study suggest that the observed changes in the microcirculation of the rectal mucosa are representative of those tissues with compromised tissue oxygenation contributing to elevated concentrations of lactate in plasma. Some patients with metabolic acidosis had only minimal obstruction of blood flow in the observed rectal capillaries (figure 3). This finding does not exclude the possibility that microcirculatory obstruction in other tissues could cause dysoxia and acidosis. Heterogeneity, in the degree of sequestration in different organs, has been observed in autopsy studies [12, 13], and it is reflected in the great variation in organ involvement in this multisystem disease. Alternatively, other factors, such as hypovolemia, hypermetabolism, or undetected comorbidity, can contribute to an increase in lactic acidosis. A weak, although significant, correlation between the depth of coma and the extent of microcirculatory obstruction in the rectal mucosa was observed in the present study. Other symptoms of severe malaria, such as the severity of anemia, were not associated with microcirculatory obstruction, and so they clearly have a different etiology.

In the present study, the case-fatality rate was high (47%) in patients with severe malaria, which mainly reflects the severity of the disease in the patients studied. The case-fatality rates, stratified in terms of level of acidosis, were not higher than those which the literature has reported for the same geographic region [24]. It should be noted that the availability of dialysis and mechanical ventilation was limited.

In conclusion, the present study directly assesses, for the first time, microcirculatory obstruction in patients with severe malaria, and it provides evidence that the degree of obstruction determines the severity of the disease, which suggests a causal connection. Therefore, the present study provides us with a tool to assess interventions aimed at improving the microcirculation, and thus reversing pathological processes, in severe malaria.

Acknowledgments

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References