Review Article

Coagulopathy in malaria

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ABSTRACT

Blood coagulation activation is frequently found in patients with malaria. Clinically apparent bleeding or disseminated intravascular coagulation (DIC) is associated with very severe disease and a high mortality. Protein C, protein S, and antithrombin levels were found to be low in *P. falciparum*, but were normal in *P. vivax* infection. Plasma levels of plasminogen activator inhibitor-1 were high in cases of *P. falciparum* infection whereas tissue plasminogen activator levels were low. Elevated plasma levels of von Willebrand factor (vWF) and vWF propeptide, thrombomodulin, endothelial microparticles have been reported in *P. falciparum*-infected patients. It has been demonstrated that severe *P. falciparum* infection is associated with acute endothelial cell (EC) activation, abnormal circulating ultralarge vWF multimers, and a significant reduction in plasma ADAMTS13 function. These changes may result in intravascular platelet aggregation, thrombocytopenia, and microvascular disease. It has also been shown that *P. falciparum*-parasitized red blood cells (pRBCs) induce tissue factor (TF) expression in microvascular ECs in vitro. Recently, loss of endothelial protein C receptor (EPCR) localized to sites of cytoadherent pRBCs in cerebral malaria has been demonstrated. Severe malaria is associated with parasite binding to EPCR. The cornerstone of the treatment of coagulopathy in malaria is the use of effective anti-malarial agents. DIC with spontaneous systemic bleeding should be treated with screened blood products. Study in Thailand has shown that for patients who presented with parasitemia ≥ 30% and severe systemic complications such as acute renal failure and ARDS, survival was superior in the group who received exchange transfusion. The use of heparin is generally restricted to patients with DIC and extensive deposition of fibrin, as occurs with purpura fulminans or acral ischemia. Antiplatelet agents interfere with the protective effect of platelets against malaria and should be avoided.

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Introduction

Malaria is the most important parasitic disease of humans, affecting more than 500 million people and causing between one and three
million deaths each year. Although malaria is mainly confined to tropical countries, cases of malaria acquired by international travellers from industrialized countries as well as immigrants from endemic countries have increased worldwide [1].

*Plasmodium falciparum* is the main cause of severe clinical malaria and death. Severe manifestations of *P. falciparum* malaria include impaired consciousness (cerebral malaria), respiratory distress, renal failure, hepatic dysfunction, profound anemia, and abnormal bleeding [2]. Many of these complications are believed to at least in part to be related to the coagulopathy and microvascular changes in this disease.

### Clinical aspects of coagulopathy in malaria

Coagulation abnormalities are frequently found in patients with severe malaria. Clinically apparent bleeding or disseminated intravascular coagulation (DIC) is associated with very severe disease and a high mortality. Bleeding in severe malaria results from several pathological processes such as thrombocytopenia, consumptive coagulopathy, and impaired clotting factor synthesis.

The reported incidence of bleeding in severe malaria has varied considerably from less than 10% to 25% [3]. In most series the incidence of hemorrhage was low, whereas in one study 83% of patients with pulmonary complications had significant bleeding [4]. In some of these studies, bleeding may have resulted from the coexistent uremia and the use of heparin or dexamethasone. Approximately 5% of adult Thai patients with cerebral malaria manifested spontaneous severe bleeding [5]. In general, bleeding usually occurs late in the course of the disease in patients with renal, pulmonary or hepatic complications and is associated with hyperparasitemia, severe anemia, thrombocytopenia and coagulopathy. DIC is observed in up to 30% of non-immune patients with severe complicated falciparum malaria [3] and indicates a poor outcome. The incidence was higher at 55% in the autopsy cases [6].

Development of symmetrical peripheral gangrene (SPG) and purpura fulminans has also been described in patients with *P. falciparum* malaria and DIC [7–9]. Several factors play a role in the development of tissue necrosis and SPG. Fibrin thrombi were found in skin biopsy specimens of patients with SPG and, postmortem, in the capillaries of various organs, suggesting DIC.

Malarial retinopathy, consisting of retinal abnormalities such as severe macular whitening and retinal hemorrhages, is a newly established diagnostic sign in severe malaria [10]. Its presence and severity are related to risk of death and length of coma in survivors. The number of retinal hemorrhages seen on fundoscopic examination correlates with the number of cerebral hemorrhages in fatal cerebral malaria. In common with cerebral hemorrhages, fibrin thrombi are seen in the small vessel at the center of hemorrhages.

### Pathophysiologic mechanisms of coagulopathy in malaria (Table 1)

#### Thrombocytopenia

Thrombocytopenia is the common feature for both *P. falciparum* and *P. vivax* malaria. The incidence of thrombocytopenia in malaria varies from 60%-80% [3]. It is more common and more severe in complicated falciparum infection. In general, thrombocytopenia alone rarely causes bleeding unless it is accompanied by coagulopathy, which is observed only in severe complicated falciparum infection. Possible causes include reduced platelet survival from peripheral destruction (by immune, consumptive, or other mechanisms), enhanced splenic uptake or sequestration, and decreased platelet production. Recently, it was shown that thrombocytopenia in early malaria is associated with VWF-mediated GP1b shedding, a process that may prevent excessive adhesion of platelets and parasitized erythrocytes [11].

#### Platelet dysfunction

During acute *P. falciparum* and *P. vivax* infection, hyperaggregation and enhanced platelet secretory activity were demonstrated [12]. This in vitro study suggested that the interaction between normal platelets and falciparum-infected erythrocytes could induce hypersensitivity of platelets, possibly through the stimulation by ADP released from infected red cells. Antibody bound to platelets as well as the invasion of platelets by malarial parasites may be other responsible mechanisms. The other aspect of platelet dysfunction during malarial infection observed in some patients is the defective aggregation of platelets in response to ADP, epinephrine and collagen but not ristocetin [12]. From electron microscopic study, circulating degranulated platelets were observed during malarial infection. The presentation of the circulating exhausted platelets as a result of persistent in vivo activation is most likely a responsible mechanism causing the platelet hypoactivity.

#### Coagulation activation

During severe complicated malarial infection, the activation of the coagulation system leading to *in vivo* thrombin generation has been demonstrated. The stimulation of the coagulation system is caused by various procoagulants present during malarial infection. The sources of the procoagulants are exposed phosphatidylserine on the cell surface of infected erythrocytes, the lysis of activated platelets together with their secretory products, and the tissue factor (TF) released from damaged vascular endothelial cells [3]. Furthermore, certain substances that are released during severe malarial infection - namely tumor necrosis factor α (TNF α) and histamine - are additional factors that promote fibrin formation [13]. The intrinsic pathway of the coagulation has also been shown to be activated in severe malaria [14]. In turn, this may cause activation of the complement system and release of bradykinin and PMN-derived elastase that could contribute to the pathogenesis of severe malaria.

Activation of the coagulation cascade also occurs in mild malaria. The degree of activation is proportional to disease severity. Several sensitive indices of intravascular coagulation, including decreased plasma antithrombin (AT) activity and increased concentrations of thrombin-antithrombin (TAT) complexes, were proportional to disease severity [15].

#### Defects in inhibitors of coagulation

Protein C, protein S, and AT levels were found to be low in *P. falciparum*, particularly in complicated cases, but were normal in *P. vivax* infection [16]. The reduction in the levels of protein C, protein S, and AT is attributed to increased consumption due to microvascular thrombosis rather than to reduced synthesis in the liver, as they correlated inversely with levels of TAT complexes. A study in Thailand showed AT levels reached 75% of normal by the 7-10th day of infection [15]. Reduction in protein C level correlated with the coma scale in cerebral malaria, as well as with the more severe clinical course of malaria, and returned back to normal after two weeks [17]. Thus, activation of

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**Table 1**

Pathophysiologic mechanisms of coagulopathy in malaria.

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protein C may be a control mechanism related to host defense in malaria as it is in sepsis.

**Impaired fibrinolysis**

Plasma levels of plasminogen activator inhibitor-1 (PAI-1) were very high in cases of *P. falciparum* infection as compared to normal controls and *P. vivax* infection [16]. This could contribute to impaired fibrinolysis. The elevation of PAI-1 also strongly correlated with a decrease in the coma scale in cerebral malaria patients and correlated inversely with a reduction in platelet count, protein C, protein S, and AT levels. Both functional and antigenic tissue plasminogen activator (tPA) levels were low. There was a good correlation between elevated PAI-1 levels with reduction in tPA levels in acute malaria. Together, these findings imply impaired fibrinolysis. Serial plasminogen activity remained within the normal range in all patients. Elevated fibrinogen degradation products (FDP) were demonstrated only in acute complicated *P. falciparum* infection. Their occurrence is mostly a compensatory mechanism secondary to increased fibrin formation during malarial infection [3].

**Cytokines**

Cytokines may play an important role in some of the pathophysiological changes in malaria. The derangement of coagulation and fibrinolysis mentioned earlier in malaria is believed to be mediated by several pro-inflammatory cytokines. The principal mediators of the activation of coagulation appear to be TNFα and interleukin 6. Serum TNFα and interleukin-6 levels were elevated in the majority of patients with *P. falciparum* infection before antimalarial treatment [17,18]. These alterations correlated significantly with the severity of the disease and with the number of circulating parasitized erythrocytes.

Parasitized erythrocytes and malarial proteins interact with macrophages in vitro and in vivo to induce production of TNFα [19,20]. TNFα has multiple effects on host response and coagulation. In vitro, TNFα elicits procoagulant effects on endothelial cells. Incubation of endothelial cells with serum from patients with severe *P. falciparum* malaria augmented expression of procoagulant activity and production of tissue factor mRNA by endothelial cells. TNFα was also found to reduce the secretion of tPA and increase the secretion of PAI-1.

**Endothelial cell activation**

Endothelial cell injury is a common feature of malarial infection and can alter hemostasis in a direct or indirect manner. The mechanisms involved in the vascular endothelial cell damage in severe complicated malaria are multifactorial. Besides the mechanical damage, the injury of these cells can be caused by toxins in the host plasma.

A significant rise in plasma levels of both von Willebrand factor (vWF) and its propeptide, indices of chronic and acute endothelial cell perturbation, respectively, has been demonstrated in patients with malaria [21]. The increased levels correlated well with parasite density and indicated endothelial damage by the parasitized erythrocytes. The highest levels were seen in cerebral malaria. Abnormal high molecular weight vWF multimers and ADAMTS13 deficiency have also been reported in association with severe malaria [22,23]. ADAMTS13 levels were evidently decreased in patients with severe malaria, compared to both patients with uncomplicated malaria and healthy controls. These findings suggest that severe falciparum infection is associated with acute endothelial cell activation, abnormal circulating ultralarge vWF multimers, and a significant reduction in plasma ADAMTS13 function, which might be contributing towards the production of the hypercoagulable state in acute malaria.

Increased numbers of circulating endothelial microparticles in patients with coma and severe malaria has also been demonstrated [24]. The mean endothelial microparticle number was significantly increased in admission blood samples only in patients with cerebral malaria, or coma and severe anemia. In patients presenting with uncomplicated malaria, or severe anemia without coma, the numbers of endothelial microparticles were not increased. This finding suggests that increased numbers of circulating endothelial microparticles could be another indicator of endothelial activation in patients with malaria complicated by coma, and endothelial microparticles may play a part in the pathogenesis of the widespread deposition of fibrin and platelets observed in fatal cases of cerebral malaria.

**Cytoadherence**

It has been demonstrated that knobs containing *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1) facilitate the cytoadherence of infected erythrocytes to vascular endothelium [25]. Cytoadherence on vascular endothelium is mediated by a number of adhesion molecules. Infected RBCs can tether and roll on several host receptors including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P-selectin. These low-affinity interactions do not by themselves lead to the arrest of the interacting cells but enhance the subsequent adhesion to CD36 or platelet GPIIV. It has also been demonstrated that falciparum infected erythrocytes are able to attach to the endothelial surface by binding platelet-decorated vWF strands in a CD36-dependent fashion, revealing a new mechanism for erythrocyte cytoadherence in malaria [26]. Early in the blood stage of *P. falciparum* infection, endothelial cells become activated to secrete ultralarge vWF (ULvWF) strands, which remain attached to the endothelial surface and rapidly bind platelets. Infected erythrocytes adhere to the platelet-decorated ULvWF strings through platelet CD36.

It has also been shown that platelet microparticles are able to bind to parasitized red blood cells (pRBCs), thereby transferring platelet antigens to the pRBC surface [27]. Platelet microparticles dramatically increase pRBC cytoadherence to human brain endothelial cells. It was concluded that platelet microparticles may participate in cerebral malaria pathogenesis while interacting with both pRBCs and human brain endothelial cells.

Severe malaria is associated with malaria parasite protein PfEMP-1 binding to endothelial protein C receptor (EPCR) [28]. Loss of EPCR in cerebral malaria localized to sites of cytoadherent pRBCs has recently been demonstrated [29].

**Tissue factor expression**

It has been shown that falciparum-infected erythrocytes induce TF expression in endothelial cells and support the assembly of multimolecular coagulation complexes [30]. It was demonstrated that mature forms of pRBCs induce functional expression of TF by endothelial cells in vitro, with productive assembly of the extrinsic Xase complex and initiation of coagulation. Late-stage pRBCs also support prothrombinase and intrinsic Xase complex formation with generation of thrombin and FXa, respectively. Postmortem brain sections obtained from falciparum-infected patients who died from cerebral malaria display a consistent staining for TF in the endothelial cells.

Sequestration of pRBCs, in addition to cytokines, microparticles, hypoxia, apoptosis, and proinflammatory molecules released by pRBCs, potentially contributes to TF expression in microvessels of the brain and in other vascular beds. The FVIIa/TF complex activates FIX and FX, generating (respectively) FXa and FXa. FXa, FVa, and prothrombin or FXa, FVIIa, and FX assemble in the membrane of activated platelets and pRBCs leading to amplification of coagulation, platelet aggregation, and inflammation.

**Treatment of coagulopathy in malaria**

The hemostatic alteration in malaria is reversed during antimalarial treatment. Therefore the cornerstone of the treatment of coagulopathy in malaria is the use of effective antimalarial agents to eradicate the
underlying infection. In Southeast Asia, quinine or artemisinin derivatives are commonly used. Full-blown DIC with spontaneous systemic bleeding should be treated with screened blood products. In patients with fluid overload, blood products can be given by exchange transfusion. A study in Thailand has shown that in patients who presented with parasitaemia > 30% and severe systemic complications such as acute renal failure and ARDS, the survival rate in the group receiving exchange transfusion was superior [31]. The amount of blood used for exchange transfusion should be at least 1.2 times the blood volume for rapid removal of parasites, toxic metabolites, procoagulants and ULVWF from the circulation as well as for supplementation of hemostatic factors and ADAMTS 13. However, the efficacy of exchange transfusion as an adjunctive treatment for severe falciparum malaria is still controversial since there has been no sufficiently powered, randomized, controlled study [32].

Corticosteroids are not indicated for thrombocytopenia. Vitamin K can be given if the prothrombin or partial thromboplastin times are prolonged. Drugs that increase the risk of bleeding (such as aspirin, corticosteroids, non-steroidal anti-inflammatory agents, and heparin) should be avoided as far as possible in patients with severe malaria [2]. The use of heparin is generally restricted to patients with DIC and extensive deposition of fibrin, as occurs with purpura fulminans or acral ischemia [33].

Recently it was shown that platelets are protective against malarial infection [34]. In vitro, purified human platelets killed P. falciparum parasites cultured in red blood cells; and inhibition of platelet function by aspirin abrogated the lethal effect human platelets exert on P. falciparum parasites. Likewise, platelet-deficient Mpl knock-out mice were more susceptible to death during erythrocytic infection with Plasmodium chabaudi. Wild type and platelet deficient mice treated with aspirin were significantly more susceptible to death. Thus, inhibition of platelet function increases susceptibility to malarial infection in mice. Platelets from a human volunteer taking aspirin for one week were unable to inhibit growth of P. falciparum parasites, implying that aspirin is potentially harmful in malarial infection. Aspirin is commonly used as an antipyretic in the developing countries. This practice might therefore be detrimental to patient with malarial infection.

Platelet factor 4 and Duffy-antigen were recently identified as key molecules in platelet-mediated killing of P. falciparum [35]. Activated platelets containing PF4-laden granules bind to pRBCs that express the Duffy-antigen receptor. Upon binding through CD36 on platelets and a parasite receptor on the red blood cell, possibly PFEMP-1, PF4 is released and binds to the Duffy-antigen receptor on red blood cells. The PF4-Duffy-antigen receptor complex translocates into the cell, co-localizes with intracellular parasites, and then kills the parasites within.

Despite the potentially critical importance of TNFs in the pathogenesis of severe complicated malaria, studies using monoclonal antibodies against TNFx had no impact on mortality [36,37]. The role of activated protein C concentrations in patients with malaria and DIC is unknown although the use of activated protein C has also been reported in patients with multi-organ failure from severe falciparum malaria [38,39]. However, this drug has recently been withdrawn due to the lack of confirmed benefit in follow up trials in severe sepsis [40].

Conclusions
Malaria is a complex syndrome. The processes of coagulation, inflammation, and pRBC sequestration are involved. A basic understanding of how these processes interact to cause the microcirculatory dysfunction and coagulopathy is needed to resolve the controversies regarding adjunctive therapy for severe malaria. It would be interesting to investigate whether therapeutics targeting TF or endothelial cells will be useful in the treatment of malaria and its complications. The development of drugs based on PF4 structure is important because of the increasing reported resistance of Plasmodium sp. to antimalarial drugs. This novel approach to treat malaria could have a significant impact on this important global health problem.

Conflict of interest statement
The author has no conflict of interest to declare.

References


