

Hematologic Changes in Malaria

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Malaria remains one of the major disease of the world, particularly in tropical countries. The infection rate of the world population was 250 million per year, and the mortality rate was 1–2 million per year.^(1,2) At present, the most important problem is the resistance of *P. falciparum* infection to anti-malarial drugs, leading to hyperparasitemia and development of serious systemic complications. In complicated *P. falciparum* infection, very high mortality rates of over 10–30% have been reported.^(3,4) Hematologic changes, which are the most common complications, play a major role in these fatal complications. These changes involve red blood cells, leukocytes, and hemostasis. They include anemia, cytoadherence of infected red cells, leukocytic changes followed by the induction of cytokines, thrombopathy and coagulopathy, particularly disseminated intravascular coagulation (DIC).

Anemia in Malaria

Anemia is one of the most common complications in malaria. The incidence of anemia in malaria was reported to be as high as 80%. Severe anemia was observed predominantly in *P. falciparum* infection with hyperparasitemia and systemic complications such as DIC. The mortality rate in malarial patients with severe anemia was 4.7% in one report⁽⁵⁾ and was even higher at 34.7% in cases with brain and lung complications.⁽⁶⁾

Mechanisms of Anemia in Malaria

Anemia in malaria may be caused by bone marrow suppression or hemolysis (Table 1).

Bone Marrow Suppression in Malaria

The evidence indicating the erythropoietic suppression occurred during malarial infection was obtained from various studies in both men and experimental animals. These lines of these evidence included the following observations. First, there were an inappropriate reticulocytosis along with erythroid hypoplasia found mostly in acute malarial infection.⁽⁷⁻⁹⁾ Second, decreased CFU-E and BFU-E in malarial bone marrow cultured with serum from infected patients⁽¹⁰⁾ together with suppression of the serum erythropoietin response to anemia^(11,12) have been observed. Finally, ineffective erythropoiesis has been demonstrated, mostly in chronic malaria.^(13,14) Various mechanisms are involved in the pathogenesis of ineffective erythropoiesis. These are dyserythropoiesis,⁽¹⁵⁻¹⁷⁾ in vitro defective heme synthesis and premature death of normoblasts.⁽¹⁸⁻²⁰⁾ In experimental animals, tumor necrosis factor (TNF) plays a role in the pathogenesis of bone marrow suppression by inducing dyserythropoiesis and reducing erythroid proliferation.⁽²¹⁾ In human

malaria, although severe anemia is associated with high levels of TNF,⁽²²⁾ the role of TNF causing suppression of CFU-E in culture has not been demonstrated.^(12,23,24) So far, the nature of the plasma inhibitors to CFU-E and BFU-E have not been identified.

Hemolysis in Malaria

Severe hemolysis occurs in hyperparasitemic *P. falciparum* infection. Hemolysis in malaria is mainly extravascular. Intravascular hemolysis occasionally occurs in certain situations and more predominantly in *P. falciparum* infection.

Extravascular hemolysis

Hemolysis of both parasitized and non-parasitized erythrocytes occurs.⁽²⁵⁻²⁷⁾ The parasitized red blood cells were destroyed by ‘pitting’ of the parasites from the red blood cells by macrophages in the spleen.⁽²⁸⁻²⁹⁾ Following the ‘pitting’ of parasites, these red cells became defective non-parasitized erythrocytes. They lose their membrane deformability

Table 1. Mechanism of Anemia in Malaria

Mechanisms	Supporting Evidence
BONE MARROW SUPPRESSION	Inappropriate Reticulocytosis
Decreased Erythroid Proliferation	Erythroid Hypoplasia Suppression of Erythroid Stem Cells in Culture with DP serum. Inappropriate Response of Erythropoietin to anemia
Ineffective Erythropoiesis	Dyserythropoiesis Premature Death of Normoblasts in Culture Defective Heme synthesis Decreased Radioactive Iron Incorporation into New RBC
HEMOLYSIS	
Extravascular	Parasitized Red Cells Sequestered in Spleen Non-Parasitized RBC Membrane Changes : lipid, peroxidation, ATP Immune Destruction Hyperactivity of Macrophages
Intravascular	Antimalarial drugs G6PD-Deficiency Malarial Fever

DP = during parasitemia

and are further destroyed in the spleen. The non-parasitized erythrocytes were destroyed by various mechanisms namely, increased activity of macrophages in spleen^(25,30) and changes of the red cell membrane caused by immune and non-immune mechanisms. Non-immune destruction occurred by various mechanisms. These were increased membrane peroxidation,⁽³⁰⁾ changes of membrane lipid and ATP, and alteration of intraerythrocytic content of Na, K, Ca.⁽³¹⁻³³⁾ Immune destruction was induced by IgM and IgG antibodies.⁽³⁴⁻³⁶⁾ However, a study in Thai malarial patients could not demonstrate the role of antibodies in hemolysis.⁽³⁷⁾

Intravascular hemolysis

Intravascular hemolysis was known as 'black water fever.' It occurs mostly in *P. falciparum* infection. Three important factors are involved in the pathogenesis of intravascular hemolysis: the use of antimalarial drugs, particularly irregular ingestion of quinine; G6PD deficiency; and malarial fever. The interaction of these three factors were found to induce intravascular hemolysis.⁽³⁸⁻⁴⁰⁾ Quinine could also cause immunohemolytic anemia and hemolytic uremic syndrome in certain cases.⁽⁴⁰⁾

Cytoadherence of Parasitized Erythrocytes

In patients who died with cerebral malaria one of the constant findings was the agglutination of red blood cells occluding the cerebral vessels, the so-called 'plugging phenomenon' (Figure 1, color page 242). The mechanisms that induce this phenomenon begin with the cytoadherence of knobs located at the membrane of parasitized red cells. The knobs contain *P. falciparum* erythrocytic membrane protein 1 (PfEMP 1).⁽⁴¹⁻⁴³⁾ The knobs adhere to receptors on endothelial cells of cerebral vessels, followed by the rosette formation and agglutination of the non-parasitized red cells,⁽⁴⁴⁻⁴⁵⁾ resulting in occlusion of cerebral vessels. These endo-

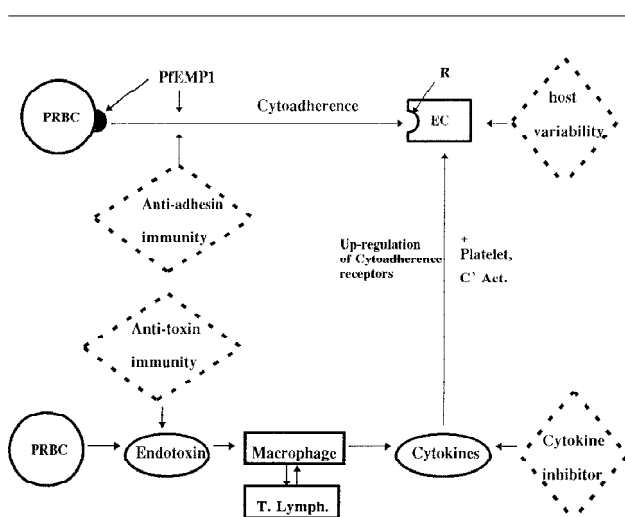


Figure 2. Mechanism and Regulation of Cytoadherence of Parasitized (*P. falciparum*) Red Blood Cell⁽⁴⁸⁾

PRBC = parasitized red blood cells

thelial cell receptors have been identified: intercellular adhesion molecules-1 (ICAM-1/CD36), thrombospondin, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), and platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31).^(41,45-47) The increase in ICAM-1 expression was observed only in non-immune *P. falciparum* infection with hyperparasitemia.^(48,49) The overexpression of ICAM-1 increased the binding of *P. falciparum*-infected erythrocytes to brain and lung capillaries and so contributes to cerebral malaria and lung complications.^(50,51) This expression was up regulated by cytokines TNF, IL-1, IL-2, IL-3^(44,52-54) and also by the activation of complement and platelets⁽⁵⁵⁾ (Figure 2). Following cytoadherence, there was sequestration of parasitized red cells to endothelial cells⁽⁵⁶⁾ leading to endothelial cell damage.^(57,58) The release of big-endothelin-1 into the circulation was observed as one indicator of this damage.⁽⁵⁹⁾ Following the endothelial cell damage, there was leakage of plasma from the circulation, hypovolemia and edema of the surrounding cells. The occlusion of the microcirculation by agglutination of parasitized and non-parasitized erythrocytes caused severe anoxia and microcirculatory stasis. This event could trigger the process of disseminated intravascular coagulation. All of these events induced pathological changes in many vital organs and occurrence of severe systemic complications particularly cerebral and lung complications.

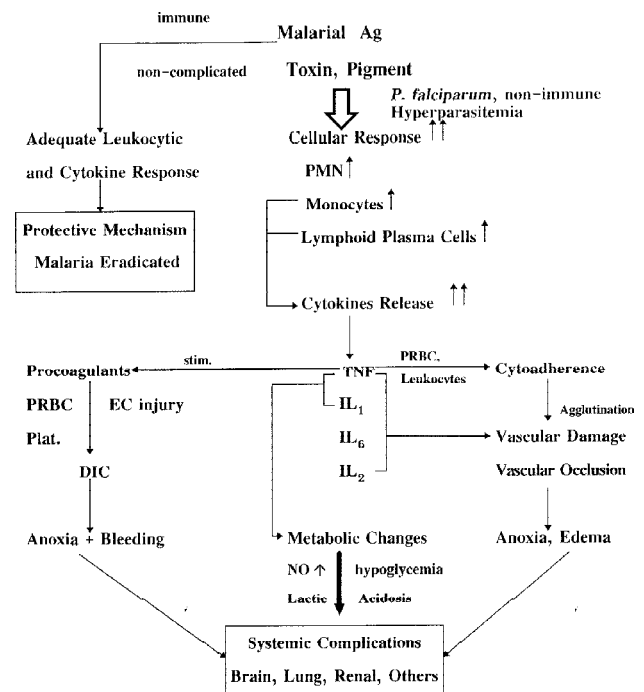


Figure 3. Leukocytic Response and Cytokines in Malaria, Role on the Protection and Induction of Complications

TNF = tumor necrosis factor, IL = interleukins, PRBC = parasitized red cells, EC = endothelial cell, plat = platelets, NO = nitric oxide, DIC = disseminated intravascular coagulation

Leukocytic Changes and Cytokines

During severe *P. falciparum* infection there are changes in leukocyte proliferation and function. The adherence of monocytes to cerebral vessels as well as neutrophils to lung vessels were observed in murine malaria.^(60,61) Following severe leukocytic proliferation, particularly of macrophages and T-lymphocytes, complement activation and release of histamine into the circulation occurred.⁽⁶²⁻⁶⁵⁾ These phenomena were closely related to severe systemic complications, thrombocytopenia and DIC.^(62,64) The circulating immune complexes consisted of malarial antigen and antibodies.⁽⁶⁵⁾ The increase in number and activity of macrophages and T lymphocytes caused the release of various soluble cytokines, namely sTNF, sIL-1, sIL-2, sIL-6 and sIFN.⁽⁵⁶⁾ These cytokines could be involved in the pathogenesis of severe systemic complications. They induced cytoadherence, vascular damage, activation of clotting system and severe metabolic changes.^(44,48,66-71) IL-1 strongly synergised with TNF involved in the induction of nitric oxide, hypoglycemia and lactic acidosis.^(66,67) These metabolic changes were the most serious complications and observed only in fatal *P. falciparum* infection.

Platelet Alteration

There were two major changes of platelet during malarial infection: thrombocytopenia and platelet dysfunction (**Figure 4**)

Thrombocytopenia

This complication has been observed in 60–80% of both *P. vivax* and *P. falciparum* infection.^(72,73) The severe degree

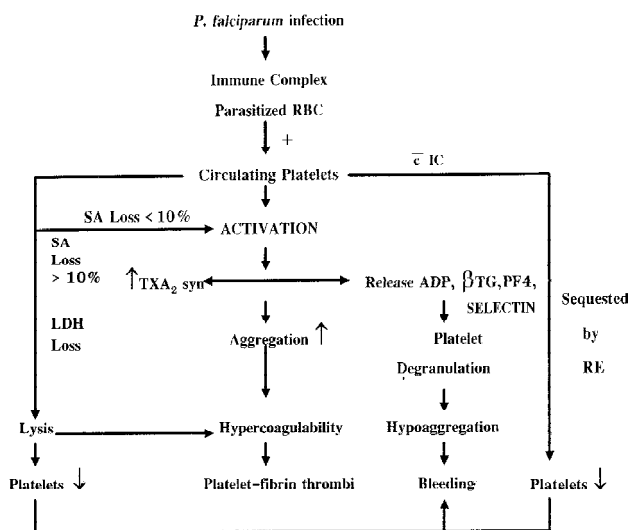


Figure 4. Alteration of Platelets in Malaria, Their Role on Bleeding and Thrombosis

IC = immune complexes, SA = sialic acid, LDH = lactate dehydrogenase, BTG = beta-thromboglobulin, PF₄ = platelet factor 4, RE = reticuloendothelial cells, TXA₂ = thromboxane A₂

and higher incidence of thrombocytopenia were observed predominantly in complicated *P. falciparum* infection.⁽⁷⁴⁾ Maximum thrombocytopenia occurred on the fifth or sixth day of infection, and gradually returned to normal within 5–7 days after parasitemia ceased.⁽⁷⁴⁾ The mechanism of thrombocytopenia in malaria is due to peripheral destruction and consumption. During malarial infection, despite the presence of thrombocytopenia, the number of megakaryocytes in the bone marrow remained adequate or increased.^(8,72) Decreased survival of platelets has been demonstrated.⁽⁷⁵⁾ The immune complexes consisted of malarial antigen and IgM or IgG antibodies with or without complement attached to platelets causing the sequestration of these injured platelets by macrophages in the spleen.^(72,75-78) Hyperactivity of macrophage also played a role on the destruction of platelets.⁽⁷⁹⁾ The activated platelets lost sialic acid from their membrane, resulting in intravascular lysis⁽⁸⁰⁾ and thrombocytopenia. Furthermore, consumption of platelets by the process of DIC was also another factor that contributed to thrombocytopenia in complicated *P. falciparum* malaria.⁽⁸¹⁾

Platelet Dysfunction

During malarial infection, two major changes in platelet function were demonstrated: platelet hyperactivity followed by platelet hypoactivity.

Platelet Hyperactivity. The evidence for hyperactivity of platelets during malarial infection comes from many studies. The lines of this evidence were hyperaggregation of platelets in response to small doses of ADP⁽⁸²⁾ (**Figure 5**) and increase in thromboxane A₂ synthesis⁽⁸³⁾ followed by the release of many substances from the platelet granules: beta-thromboglobulin, platelet factor 4, and selectin.^(84,84a) During malarial infection, platelets were stimulated by various factors: immune complexes, surface contact of platelet membrane to malarial red cells (**Figure 6**),⁽⁸⁵⁾ and damage to endothelial cells. These stimulated platelets lost sialic acid from their membrane and became hyperactive. The lysis of

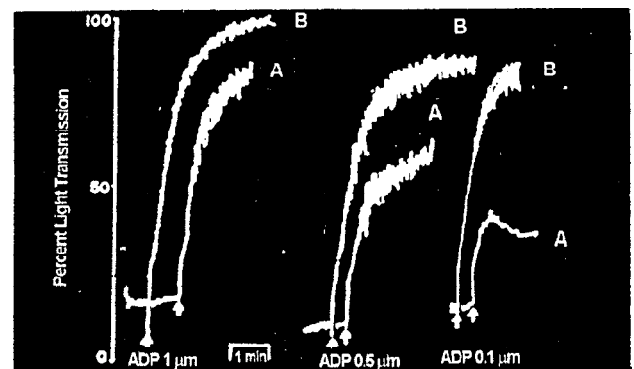


Figure 5. Hyperaggregation of Platelets during Malarial Infection Stimulated with ADP 1 μ, 0.5 μm, 0.1 μm⁽⁸²⁾

A = normal, B = *P. falciparum* infected patient

these injured platelets could occur intravascularly.⁽⁸⁰⁾ The intravascular lysis along with hyperaggregation accompanied by the increased release of various substances from the platelet granules induced platelet-fibrin thrombi formation as well as disseminated intravascular coagulation.

Platelet Hypoactivity was demonstrated by decreased platelet aggregation on stimulation by various substances such as ADP, epinephrine and collagen.^(74,82) This abnormality occurred transiently and returned to normal within 7 to 14 days after parasitemia ceased (**Figure 7**). As mentioned above, the hyperactive platelets released many sub-

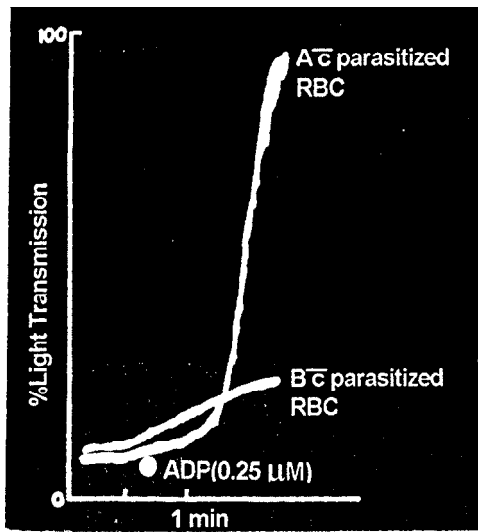


Figure 6. ADP-Induced Platelet Aggregation in Parasitized RBC and Normal RBC⁽⁸⁵⁾

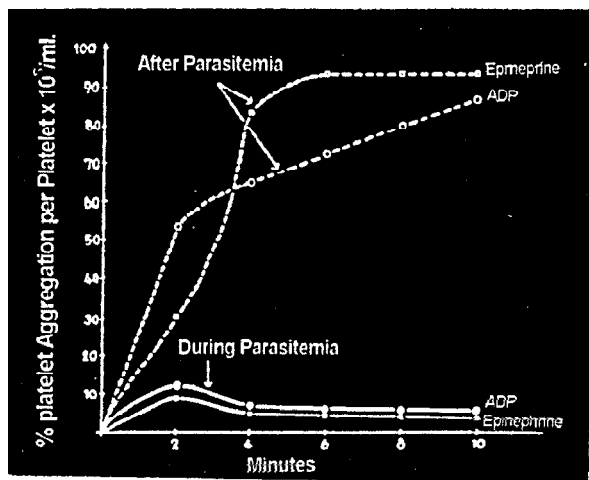


Figure 7. Platelet Aggregation during and after Parasitemia⁽⁷⁴⁾

21-year-old mal, *P. falciparum* infected rate 40% with CNS, renal, bleeding, DIC complications

stances from their granules, resulting in degranulated circulating platelets. The degranulated platelets became exhausted and lost their function. They became hypoaggregated to various stimulators. The severe hypoaggregation along with severe thrombocytopenia could contribute to the pathogenesis of bleeding in malaria.

Disseminated Intravascular Coagulation

DIC is the most serious hematologic complication in malaria. The incidence of DIC was observed to be around 10–30%.^(73,86-87) It occurs only in non-immune, hyperparasitemic *P. falciparum* patients with severe systemic complications. The mortality rate of *P. falciparum* infection with DIC and severe systemic complications was reported to be as high as 42–75%.^(81,88)

Evidence for DIC in complicated *P. falciparum* infection has been obtained from many studies. First, the rapid clearance of radioactive fibrinogen was demonstrated in patients with cerebral malaria.^(89,90) Second, the hemostatic derangement consisted of thrombocytopenia simultaneously with prolonged partial thromboplastin time and/or prothrombin time along with increased D-dimer or fibrinogen degradation products were also observed.^(88,91-93) Finally, disseminated fibrin thrombi in various vital organs were demonstrated in the patients who died with complicated *P. falciparum* infection.^(81,94,95)

Mechanisms of DIC (Figure 8) During complicated *P. falciparum* infection activation of the clotting system resulted in thrombin generation and intravascular coagulation.^(96,97) Various stimulators are involved in the process of activation. Procoagulants came from the alteration of parasitized red blood cell membrane^(98,99,100), as well as intravascular lysis of red blood cells and platelets.⁽⁸⁰⁾ Tissue factor

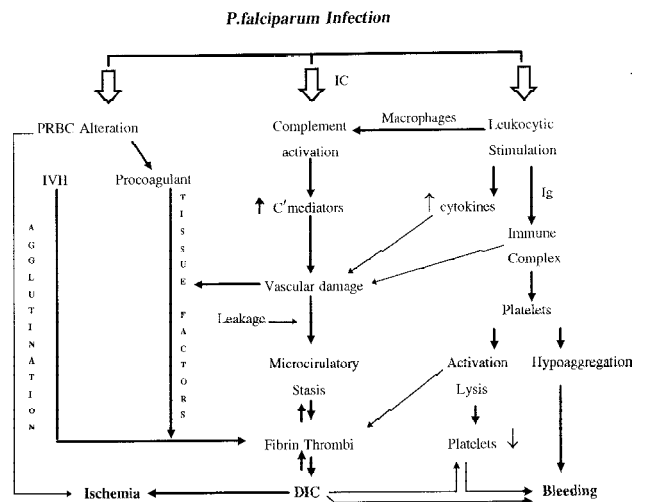


Figure 8. Pathogenesis of DIC in Complicated *P. Falciparum*.

PRBC = parasitized red blood cells, IVH = intravascular hemolysis, C' = complement, IC = immune complexes

was released into the circulation by activated monocytes⁽¹⁰¹⁾ and damaged endothelial cells. The complement activation along with increase in many soluble cytokines, particularly TNF and IL-1, also contributed to the process of intravascular coagulation. Finally, microcirculatory stasis, which occurs as an end result of vascular damage, is another important factor that contributes to DIC.

Role of Hematologic Changes on the Occurrence of Systemic Complications

Three major hematologic changes play a role in the pathogenesis of systemic complications. These are red blood cell alterations, leukocytic changes, and hemostatic derangement, namely thrombopathy and coagulopathy. Some of the changes in the erythroid system cause anemia (from bone marrow suppression and hemolysis), accompanied by cytoadherence and agglutination of malarial red blood cells in microvessels. Both events cause anoxia of the vital organs. The proliferation of leukocytes induces a hyperimmune reaction and release of many soluble cytokines. Both the hyperimmune reaction and cytokines could cause endothelial cell damage as well as activate thrombin generation, leading to the process of DIC. Platelet alteration along with DIC could cause severe bleeding. All of these events—anoxia, DIC, bleeding, microcirculatory stasis and severe metabolic changes that were mainly induced by IL-1 in synergy with TNF—bring on severe systemic complications. It is clearly understood that the most important factor that induces all of these changes is the virulence of malaria infection in hyperparasitemic *P. falciparum* infection. The most important treatment is therefore to eradicate the

hyperparasitemia immediately and effectively along with other supportive measures. Besides the prompt administration of effective antimalarial drugs such as quinine and artesunate, exchange transfusion has been reported to be an effective adjunctive treatment in severe complicated *P. falciparum* infection.^(4,102-107) The rationale for exchange transfusion are rapid clearance of parasites from the circulation, replacement of the new red cells to improve rheology and tissue oxygenation, and removal the soluble cytokines and procoagulants from the circulation. Although there was some improvement in the survival rate from this treatment, the overall mortality rate is still high. This remains an important problem at the present time.

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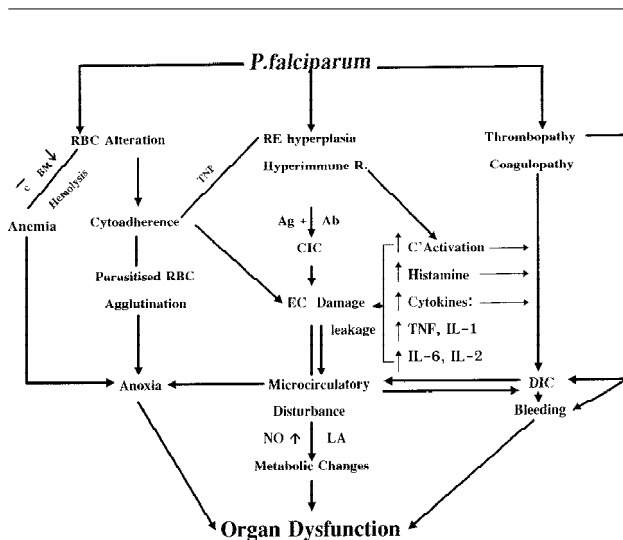


Figure 9. Hematologic Changes and Their Role on the Occurrence of Systemic Complications in *P. falciparum* Infection

BM = bone marrow, NO = nitric oxide, LA = lactic acidosis, C' = complement, TNF = tumor necrosis factor, IL = interleukins, CIC = circulating immune complex

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