Hemolysis-associated hypercoagulability in sickle cell disease: the plot (and blood) thickens!

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While sickle cell disease and other chronic hereditary and acquired hemolytic anemias are considered hypercoagulable states, a unifying mechanism explaining the hemostatic activation has been elusive.[1] Patients with sickle cell disease exhibit increased thrombin and fibrin generation,[2,3] increased tissue factor activity,[4] increased basal and stimulated platelet activation,[3,5-8] and manifest clinical thrombotic complications, including pulmonary emboli, *in situ* pulmonary thrombosis, and stroke.[9-16]

In their study published in this issue of the journal, Ataga *et al*. examine the associations of measures of pulmonary hypertension, defined by increases in the estimated pulmonary artery systolic pressure by transthoracic Doppler echocardiography, with measures of coagulation activation, inflammation and endothelial activation in 76 patients with sickle cell disease.[17]

Surprisingly, monocyte counts and markers of inflammation were not associated with hemostatic indices, while measures of hemolytic rate (hemoglobin, total and indirect bilirubin, and lactate dehydrogenase) correlated with indices of hypercoagulability. In fact, hemoglobin levels were inversely correlated and lactate dehydrogenase values directly correlated with all measures of hemostatic activation, including thrombin-antithrombin complex (TAT), prothrombin fragment F1+2, D-dimer, sCD40L (a marker of platelet activation), and soluble VCAM-1 (a marker of endothelial activation).

These results are similar to those of our recent studies exploring the mechanisms of platelet activation in sickle cell disease. Using flow cytometric assessment of platelet glycoprotein IIbIIIa and cell surface P-selectin expression, Villagra *et al*. found that platelets were activated in steady state sickle cell disease.[8] Similar to the findings of Ataga *et al*., the platelets were further activated in patients with pulmonary hypertension, and this activation correlated directly with measures of hemolytic rate, such as low hemoglobin and high reticulocyte counts. From a mechanistic standpoint, direct exposure of platelets to cell free hemoglobin *in vitro* resulted in activation. While platelet activation was inhibited by nitric oxide donors as expected, the nitric oxide inhibitory effect was abolished by inclusion of pathophysiologically relevant levels of cell free hemoglobin in the platelet-nitric oxide donor mixture.[8]

These studies are consistent with our increased knowledge of a novel mechanism of disease, hemolysis associated endothelial dysfunction and vasculopathy.[18-21] During normal physiology, endothelial-derived nitric oxide is protected from the scavenging effects of intracellular hemoglobin via the formation of nitric oxide diffusional barriers in the unstirred layer around the erythrocyte membrane and the cell free zone that forms along endothelium in laminar flowing blood.[22-26] These combined diffusional barriers reduce the reaction rate of nitric oxide with oxy- and deoxy-hemoglobin by up to 1,000 fold. Furthermore, robust scavenging and vascular protection systems exist to detoxify plasma hemoglobin via the haptoglobin, CD163, hemoxygenase, biliverdin reductase, and $p21^{WAF-1/CIP-1}$ pathway.[21] During intravascular hemolysis, these diffusional barriers are disrupted and the scavenging systems overwhelmed, resulting in the accumulation of cell free plasma hemoglobin which quenches nitric oxide and generates reactive oxygen species. In addition, arginase I is released from the red blood cell during hemolysis and metabolizes arginine, the substrate for nitric oxide synthesis, further impairing homeostasis.[20]

In addition to regulating vascular tone and inhibiting endothelial adhesion molecule expression, nitric oxide has potent antithrombotic effects. Via cGMP-dependent signaling, nitric oxide inhibits platelet activation.[8,27-29] Nitric oxide has also been shown to inhibit tissue factor expression,[30,31] although there are conflicting data on this.[32] Besides nitric oxide scavenging by plasma hemoglobin, hemolysis is also associated with phosphatidylserine exposure on red cells which can activate tissue factor and form a platform for coagulation.[33,34]

Interestingly, this pathway may mechanistically explain the link between splenectomy (surgical and autosplenectomy) and pulmonary hypertension and thrombosis.[35-42] An important function of the spleen is to clear senescent, oxidized and phosphatidylserine-exposing red cells and thus limit intravascular cell microvesiculation, hemolysis and phosphatidylserine exposure.[33,43,44] Increases in the plasma concentration of cell free hemoglobin and red cell microparticles after splenectomy could paradoxically increase nitric oxide scavenging, vascular injury and thrombosis, despite increasing hemoglobin levels. Interestingly, since priapism is also now recognized as a complication of hemolytic anemia and low nitric oxide bioavailability,[45-53] an increase in intravascular cell free plasma hemoglobin and red cell microparticles after splenectomy could also explain the observed development of priapism after splenectomy.[54,55]

The study by Ataga *et al*.[17] now clearly links multiple indices of hypercoagulability with both hemolysis and progressive vasculopathy, characterized by pulmonary hypertension. We, therefore, propose that the thrombophilia and hemostatic activation common to most hemolytic conditions,[56-59] including paroxysmal nocturnal

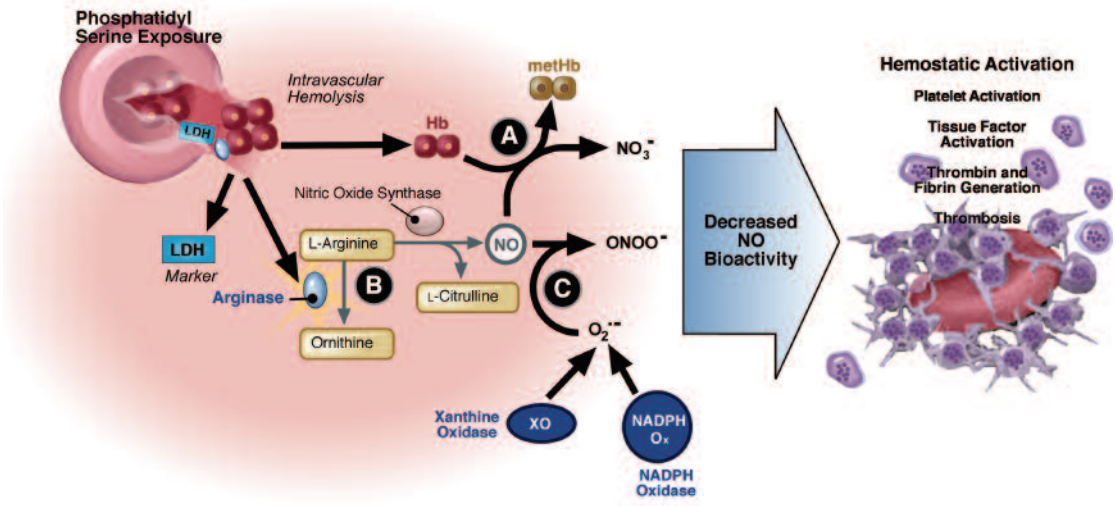

Figure 1. Hemolysis-associated hemostatic activation. Intravascular hemolysis releases hemoglobin into plasma which quenches NO and generates reactive oxygen species (directly via fenton chemistry or via induction of xanthine oxidase and NADP oxidase). In addition, arginase I is released from the red blood cell during hemolysis and metabolizes arginine, the substrate for NO synthesis, further impairing NO homeostasis. The depletion of NO is associated with pathological platelet activation and tissue factor expression. Hemolysis and splenectomy are also associated with phosphatidylserine exposure on red cells which can activate tissue factor and form a platform for coagulation.

hemoglobinuria, sickle cell, thalassemia, red cell membrane disorders, red cell enzymopathies, thrombotic thrombocytopenic purpura, malaria, cardiopulmonary bypass, transfusion of aged blood, and alloimmune hemolysis, may be explained by the very feature they all share, intravascular hemolysis (Figure 1). Evaluation of this hypothesis may help identify novel approaches for these numerous conditions, including nitric oxide donors, nitrite (which can *harness* hemoglobin as an nitric oxide generator),[60-63] hemoglobin scavengers, and anti-hemolytic therapies.[64]

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