Thrombosis and Sickle Cell Disease

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ABSTRACT

Sickle cell disease (SCD) is characterized by the presence of sickle hemoglobin, which has the unique property of polymerizing when deoxygenated. The pathophysiology of acute and chronic clinical manifestations of SCD have shown the central role of dense, dehydrated red cells in acute and chronic clinical manifestations of this pathology. Recent studies have indicated that SCD is characterized by a hypercoagulable state that contributes to the vaso-occlusive events in microcirculation, leading to acute and chronic sickle cell–related organ damage. This review discusses, in the context of SCD, (1) abnormalities in the coagulation system, (2) perturbation of platelet activation and aggregation, (3) vascular endothelial dysfunction, (4) the contribution of cell inflammatory responses, and (5) the connection with nitric oxide metabolism. We also review the available studies on the therapeutic approaches in clinical management of hypercoagulability in SCD.

KEYWORDS: Hypercoagulability, vascular endothelial dysfunction, inflammation, neutrophils, dense red cells

Sickle cell disease (SCD; Online Mendelian Inheritance in Man [OMIM] No. 603903) is an autosomal recessive genetic red cell disorder with a worldwide distribution that results from a point mutation (β^S, 6V) in codon 6, with the insertion of valine in place of glutamic acid, leading to the production of a defective form of hemoglobin (hemoglobin S [HbS]). In the United States ~75,000 people have SCD. In Europe, immigration from developing countries has increased the prevalence of SCD through the second half of the 20th century, and now almost 20,000 to 25,000 SCD patients have been registered.[1–4] Sickle hemoglobin (HbS) shows peculiar biochemical properties, polymerizing when deoxygenated. Studies of the kinetics of HbS polymerization following deoxygenation have shown that the kinetics of polymer formation is a high-order exponential function of hemoglobin concentration, thus demonstrating the crucial role of cellular HbS concentration in the phenomenon of sickling.[5,6]

HbS polymerization is associated with a reduction in cell ion and water content (cell dehydration), increased red cell density, and further acceleration of HbS polymerization.[5–7] Pathophysiological studies have shown that the dense, dehydrated red cells play a central role in acute and chronic clinical manifestations of SCD, in which intravascular sickling in capillaries and small vessels leads to vaso-occlusive and impaired blood flow.[6,8] The persistent membrane damage associated with HbS polymerization also favors the generation of distorted rigid cells and further contributes to vaso-occlusive events and cell destruction in the peripheral circulation. These damaged dense sickle red cells also show a loss of phospholipid asymmetry with externalization of phosphatidylserine (PS; Fig. 1), which is believed to play a significant role in promoting macrophage recognition with removal of

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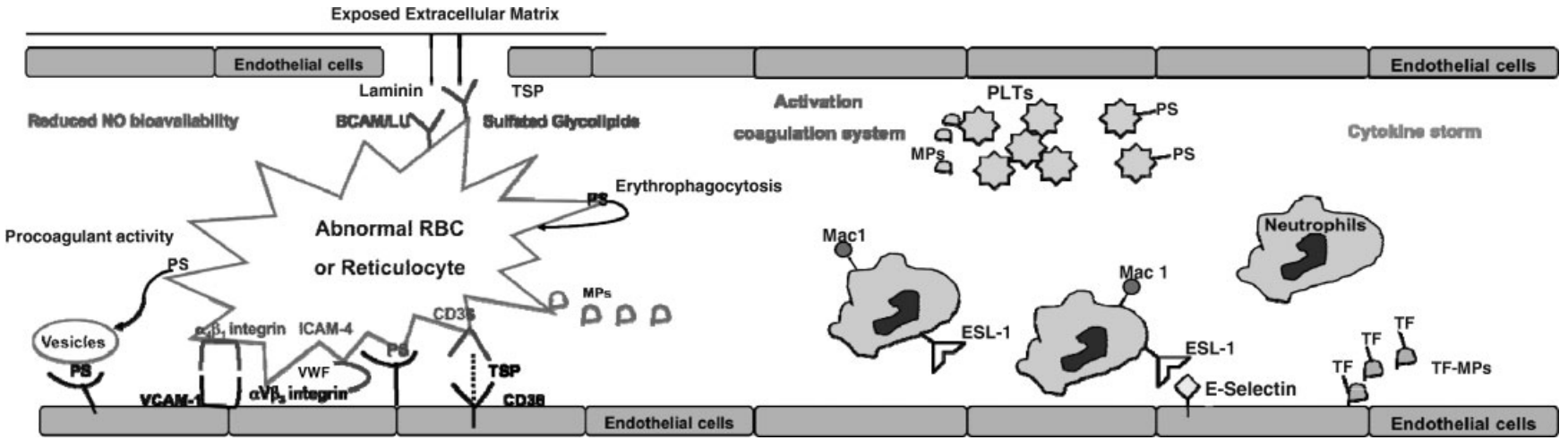

Figure 1 Schematic diagram of mechanisms of acute sickle cell vaso-occlusive events involving the adherence of sickle red blood cells (RBCs) or reticulocytes and neutrophils to the abnormally activated endothelial cells with the participation of activated and phosphatidyl-serine (PS)-rich platelets (PLTs), activation of the coagulation system, and a resulting cytokine storm. BCAM/LU, Lutheran blood group protein; ESL, neutrophil E-selectin ligand; ICAM-4, Landsteiner-Weiner (LW) blood group glycoprotein; Mac1, β2 integrins ($\alpha_M\beta_2$ or CD11b/CD18); MPs, microparticles; NO, nitric oxide; TSP, thrombospondin; VWF, von Willebrand factor.

erythrocytes (erythrophagocytosis) and activation of coagulation. Setty et al have recently shown that increased PS-exposing red cells are associated with thrombin generation as well as increased tissue factor (TF) expression, more likely due to the increased circulating hemoglobin than as a direct connection between PS-exposing red cells and vascular endothelium.[9–11]

A complex perturbation of hemostasis has been reported in SCD both under steady state and during acute events. The vaso-occlusive events in the microcirculation result from a complex and still partially known scenario involving the interactions among different cell types, including dense, dehydrated sickle cells, reticulocytes, abnormally activated endothelial cells, leukocytes, platelets, and plasma factors such as coagulation system, cytokines and oxidized proinflammatory lipids.[12–20] Clinical manifestations of the prothrombotic state of sickle cell patients include venous thromboembolism, in situ thrombosis, and stroke, associated with an higher risk of thrombotic complications in patients splenectomized or with functional hyposplenism.[6,21–27]

COAGULATION SYSTEM AND SICKLE CELL DISEASE

Studies in SCD have shown increased prothrombin fragment 1.2 (F1.2), thrombin-antithrombin complexes, plasma fibrinogen products, D-dimer, and decreased factor V, suggesting an enhanced thrombin generation and supporting a chronic thrombophilic state in SCD patients that is further amplified during acute events (Table 1).[13,17,28–38] Sickle cell patients also show abnormal (decreased) levels of factor (F) VII and activated FVII compared with normal subjects, most likely due to increased TF activity that promotes accelerated FVII turnover.[37,39] Moreover, in sickle cell patients under steady state conditions, decreased FXII and FIX have been observed, possibly related to activation of the intrinsic coagulation pathway[40] (Table 1).

Ataga et al recently reported high levels of thrombin-antithrombin complex, prothrombin fragment F1 + 2, and D-dimer, associated with an activation profile of vascular endothelium (i.e., soluble vascular endothelial cell adhesion molecule) in sickle cell subjects with pulmonary hypertension compared with normal controls.[41] It is interesting to note that these authors also observed a correlation between the rate of hemolysis and the hypercoagulability state in SCD patients with pulmonary hypertension.[41] However, in another cohort of SCD patients with mild pulmonary hypertension, van Beers et al reported no association of the hypercoagulative state of SCD with the early phase of pulmonary hypertension,[42] suggesting that more complex events are involved in the pathogenesis of pulmonary hypertension in SCD.

Table 1 Platelets and Coagulation System in Sickle Cell Disease

Platelet Parameters and Coagulation System	Alterations	References
Platelet activation (CD62, CD63, GPIIb/IIIa)	Increased	Tomer et al,[29,36] Foulon et al,[64] Browne et al,[65] Wun et al,[69] Famodu and Oduwa,[70] Lee et al[71]
Platelet aggregation	Increased	Kenny et al,[66] Westwick et al,[67] Winichagoon et al[68]
Phosphatidyl serine–rich platelets	Increased	Tomer et al[29,36]
Thrombin-antithrombin complex	Increased	Peters et al,[28] Rickles and O'Leary,[31] Stuart and Setty,[32] Green and Scott,[33] Richardson et al,[35] Tomer et al,[36] Kurantsin-Mills et al,[37] Ataga et al,[41] van Beers et al[42]
Prothrombin fragment F1 + 2	Increased	Peters et al,[28] Tomer et al,[36] Ataga et al,[41] van Beers et al[42]
Plasmin-antiplasmin complex	Increased	Tomer et al[29,36]
FV	Decreased	Leslie et al[30]
FVII and FVIIa	Accelerated turnover	Kurantsin-Mills et al,[37] Hagger et al[39]
FXII and FIX	Decreased	Branch and Rodgers[40]
Fibrinogen and fibrin-fibrinogen complex	Increased	Adam et al,[17] Leslie et al[30]
Fibrinopeptide A	Increased	Green and Scott,[33] Kurantsin-Mills et al,[37] Westerman et al[38]
D-dimer	Increased	Adam et al,[17] Ataga et al,[41] van Beers et al,[42] Francis and Haywood[120]
Protein C and S	Decreased	Green and Scott,[33] Westerman et al,[38] Tam,[45] el-Hazmii et al[46] Karayalcin and Lanzkowsky,[46] Kuypers et al,[48] Lane et al,[49] Francis and Haywood[120]
Plasminogen activator inhibitor	Increased	Tomer et al,[36] Westerman et al,[38] Nsiri et al[50,51]

GP, glycoprotein.

A possible role in activating the clotting system with thrombin generation in sickle cell patients is also played by the circulating TF-positive microparticles (MP) derived from red cells, platelets, endothelial cells, and monocytes (Fig. 1). Shet et al reported increased TF-positive MPs in sickle cell patients in both steady state and during acute events compared with normal controls, thereby suggesting a possible contribution of TF-positive MPs to the sickle cell prothrombotic state.[43] In a 2009 study, van Beers et al observed higher levels of circulating MPs from erythrocytes and platelets in SCD patients under steady state compared with normal controls, further increasing during acute vaso-occlusive events.[44] These authors did not find TF-MPs as previously reported by Shet et al,[43] but they observed that the levels of circulating MPs strongly correlate with hemolysis, von Willebrand factor (VWF), D-dimer, and F1 + 2 levels, supporting a role of MPs in the prothrombotic state of sickle cell patients.[44]

Studies on natural anticoagulant in patients with SCD showed low levels of protein C and S, suggesting a possible perturbation in either their synthesis related to liver disease or a consumption by increased TF and thrombin production. The relative deficiency of protein C and S was reported to have a clinical impact on the risk of developing stroke in children with SCD.[33,34,38,45–47] However, variable levels of protein S and C were observed in sickle cell patients during acute events compared with steady state, suggesting a more complex biological scenario.[33,34,38,45–47] In addition, the increased percentage of circulating sickle red cells exposing PS might bind protein S, most likely contributing to protein S reduction.[48,49]

The prothrombotic state of SCD is also associated with abnormalities in the fibrinolytic system, mainly characterized by increased plasma levels of plasminogen activator inhibitor (PAI)-1 in both steady state and during sickle acute events compared with the normal population.[36,38,50,51] Because the synthesis of PAI-1 is increased in activated or damaged endothelial cells and also secreted by activated platelets,[52] the increased PAI-1 levels in sickle cell patients suppressing the normal fibrinolytic system might participate in the pathogenesis of vaso-occlusive events in SCD. Although studies have been performed on coagulation system activation during acute events in SCD, inconclusive data have been reported thus far.[34,36,38,53,54]

Studies on thrombophilic deoxyribonucleic acid mutations have been performed in patients with SCD to assess their possible impact on sickle cell thrombotic events. Factor V Leiden and the prothrombin variant (FII G20210A) have been evaluated in sickle cell patients of African descent. Because the frequency of these two alleles is low in the African descendent population, the contribution of these two thrombophilic mutations on thrombotic clinical manifestation of SCD seems to be limited.[55–58] However, in sickle cell patients from eastern Saudi Arabia and Lebanon, a nonsignificantly higher frequency of FII G20210A was observed compared with normal controls.[59] Moreover, an association between factor V Leiden and venous thrombotic events was shown in Iranian patients with SCD, supporting a different impact of this thrombophilic mutation within sickle cell patients from different ethnic groups.[60,61]

Studies on methylene tetrahydrofolate reductase (MTHFR) polymorphisms were performed in patients with SCD, but no association between any MTHFR mutation and thrombotic events was highlighted.[55,57,62,63]

PLATELETS AND SICKLE CELL DISEASE

Studies on the role of platelets in clinical manifestations of SCD on both steady state and acute events have been partially characterized, and much still remains to be investigated (Table 1). Increased production of thromboxane-A2 and prostaglandin metabolites associated with decreased platelet trombospondin-1 levels, suggesting a chronic activation of platelets, was shown in urine from SCD patients.[64,65] Other studies also showed increased platelet aggregation in SCD.[66–68] Increased platelet activation markers such as P-selectin (CD62), CD63. and activated glycoprotein (GP)IIb/IIIa as well as increased plasma soluble factors as platelet factor (PF)-3, -4 and β-thromboglobulin and platelet-derived soluble CD40 ligand (sCD40L) were reported in SCD patients using a cytofluorimetric approach.[36,69–71] Villagra et al suggested that the relative reduction in nitric oxide (NO) bioavailability might participate in triggering platelet activation as well as increasing platelet adhesive properties in patients with SCD.[72] PS-rich platelets have also been described in SCD patients; these show an increased binding to annexin V that might participate in activation of the coagulation system.[36] Recently, Proença-Ferreira et al reported an increased ability of platelets from subjects with SCD to adhere to fibrinogen through modulation of platelet's cyclic adenosine monophosphate content via phosphodiesterase-3A activity associated with increased $\alpha_{IIb}\beta_3$-integrin activation.[73] It is interesting to note that in clinical management of SCD patients during acute pain events, the platelet count first decreases, followed by a paradoxical increase associated with higher plasma levels of the platelet products PF4 and β-thromboglobulin (βTG). This suggests a further amplification of platelet activation during acute events.[33,74–76] The reduction in platelet content and then the rebound during resolution of acute events have been related to functional asplenia of patients with SCD.[13,15,65]

Studies on polymorphisms of human platelet alloantigen (HPA), a complex of platelet glycoproteins with other cell-bound factors, showed a possible pro-

thrombotic role in different thrombotic disorders and in sickle cell patients with cerebrovascular events.[77–80] HPA polymorphisms result in platelet structural changes and/or levels of adhesion proteins. In a case-control study, Al-Subaie et al reported that the HPA-3 variant, which has an isoleucine-to-serine substitution close to the C-terminus of the GPIIb heavy chain, seems to be an independent risk factor for acute vaso-occlusive events in SCD (Table 2).[81]

However, no conclusive evidence is actually available on the real impact of thrombotic mutations in SCD mainly due to the limited number of studies and the differences in the genetic background of the sickle cell population studied.

VASCULAR ENDOTHELIUM DYSFUNCTION IN SICKLE CELL DISEASE

SCD patients have shown abnormally activated circulating endothelial cells that increase during acute vaso-occlusive crisis, which is compatible with the presence of chronic vascular endothelial damage further worsening during acute events.[8,54,82–85] Recent studies on the sickle cell-endothelium adhesive mechanism identified different interactions that may have particular relevance in the generation of acute vaso-occlusive events: (1) the integrin α $\alpha_{4b}\beta_1$ receptor of fibronectin and VCAM-1, E-selectin, and P-selectin; (2) the thrombospondin (TSP) and/or collagen and receptor CD36, present on the surface of endothelial cells, platelets, and reticulocyte-rich subpopulations of normal and sickle erythrocytes; (3) the sulfate glycolipids, which bind TSP, VWF multimer, and laminin;[6,86,87] (4) the Lutheran blood group proteins (BCAM/LU), whose expression is increased in red cells from SCD patients as is their binding to the α5 subunit of laminin, a component of extracellular subendothelial matrix;[88,89] (5) the ICAM-4 (Landsteiner-Weiner blood group glycoprotein), which binds $\alpha V\beta 3$ integrin receptors on endothelial cells;[90–93] and (6) the exposure of PS, detectable in a subpopulation of sickle red cells, which participates in sickle cell adhesion to activated endothelium[9,94–97] (Fig. 1).

In SCD patients, increased levels of VWF and, in particular, large VWF multimers were observed and associated with acute vaso-occlusive events.[98–101] The increased levels of circulating VWF multimers are related to the activity of the metalloprotease ADAMTS 13 (a disintegrin and metalloproteinase with thrombospondin domain 13) that cleaves the hyperadhesive ultra-large VWF under conditions of high fluid shear stress,[102–104] playing an important role in maintaining the endothelial cell surface free from hyperadhesive ultra-large VWF.[105] Studt et al showed that free hemoglobin can inhibit ADAMTS 13 activity, affecting the VWF cleavage in patients with thrombocytopenic purpura.[106] Schnog et al[98] reported a decreased ADAMTS 13 activity in

Table 2 Genetic Modifiers of Platelet Activation and Endothelial Function with Effects in Sickle Cell Disease Acute Vaso-Occlusive Events

Genetic Modifier	Target	Effects	References
Human platelets alloantigen (HPA) polymorphism: HPA-3 variant	C-terminus of GPIIb heavy chain on platelets	Independent risk factor for acute vaso-occlusive events	Al-Subaie et al[81]
G1238C VCAM-1 single nucleotide polymorphism	Modulation of vascular endothelial adhesion molecule	Protective effects from stroke in SCD children	Taylor et al[108]
TNF-α and IL-4 polymorphism	Proinflammatory cytokines	Linked to risk of stroke in SCD children	Hoppe et al[109]
Endothelin-1 (ET-1) polymorphism: ET-1 T8002C	Vascular active molecule	Increased susceptibility to acute chest syndrome	Chaar et al[110]
Endothelium nitric oxide synthase (eNOS) polymorphism: T-786C variant	Endothelial NO metabolism	Reduced susceptibility to develop acute chest syndrome in SCD children Increased susceptibility to develop acute chest syndrome in female patients with SCD	Chaar et al[110] Sharan et al[111]
Klotho (KL) gene polymorphism	β-Galactosidase like proteins involved in endothelial NO homeostasis	Linked to stroke, osteonecrosis, leg ulcers, and priapism	Baldwin et al,[112] Sebastiani et al,[113] Nolan et al[114]
Receptor tyrosine kinase Tie polymorphism	Kinase involved in signaling pathways for chemotaxis and NO metabolism	Linked to leg ulcers	Nolan et al[115]
Polymorphism of proteins from bone morphogenic protein pathway	Proteins involved in signaling pathway to protect vascular endothelial surface	Linked to risk factor for stroke, priapism, leg ulcers	Baldwin et al,[112] Sebastiani et al,[113] Elliott,[116]

SCD, sickle cell disease; GP, glycoprotein; VCAM, vascular cell adhesion molecule; TNF, tumor necrosis factor; IL, interleukin; NO, nitric oxide.

sickle cell patients compared with normal controls as well as an associated reduced ADAMTS 13-to-VWF antigen ratio.[99,107] The same authors further demonstrated an inverse relationship between ADAMTS 13 activity and extracellular hemoglobin levels, suggesting that hemoglobin might compete with VWF for ADAMTS 13. This would cause a relative deficiency of ADAMTS 13, which in turn would block the proteolysis of VWF and lead to the accumulation of hyperadhesive ultra-large VWF on the vascular endothelium surface.[98,99,107] This mechanism might represent a new element contributing to the complex scenario of microvascular thrombosis in SCD.

Genetic modifiers with functional effects on vascular endothelium homeostasis were evaluated in few studies in patients with SCD (Table 2). Taylor et al showed a protective effect from stroke of G1238C *VCAM1* single nucleotide polymorphism in sickle cell patients.[108] Polymorphisms of cytokines involved in proinflammatory responses, such as tumor necrosis factor (TNF)α and interleukin (IL)4, were correlated with the risk of stroke in children with SCD.[109] Polymorphisms of either endothelin-1 (*ET-1*) and endothelial-NO synthase (*eNOS*) were linked to the susceptibility of sickle cell patients to acute vaso-occlusive events.[110,111] Gene polymorphisms in Klotho (*KL*), encoding for a β-glucosidase-like protein involved in endothelial NO homeostasis, were linked to stroke and osteonecrosis in SCD as well as to leg ulcers.[112–115] A polymorphism of the receptor tyrosine kinase Tie, a factor involved in NO metabolism and leukocyte chemotaxis, was identified as a possible additional genetic modifier associated with leg ulcers in SCD patients.[112–115] Finally, polymorphisms of genes from bone morphogenic protein signaling pathway involved in protection of the vascular endothelial surface were associated with the risk for different acute vaso-occlusive events including stroke, priapism, or leg ulcers.[112,113,116] Although these studies show the possible role of genetic modifiers of endothelial homeostasis affecting acute vaso-occlusive events in SCD, further investigations should be performed to verify the real impact of these gene polymorphisms on larger SCD patient populations.

INFLAMMATION AND LEUKOCYTES PARTICIPATE IN SICKLE CELL VASO-OCCLUSIVE EVENTS

A chronic inflammatory state has been described in SCD patients characterized by increased plasma levels of acute phase proteins and soluble cytokines such as IL1β, IL6, TNF-α, and endothelin-1 (ET-1) that are further elevated during acute vaso-occlusive events. These factors participate in leukocyte chemotaxis, modulate vascular tone, and contribute to sickle cell–related tissue damage.[18,20,85,117–122] Recently, Enenstein et al reported an altered ratio of proinflammatory factor RelA to anti-inflammatory factor KLF2 in sickle cell children with high risk of stroke, thus suggesting a complex prothrombotic network involving abnormal activated vascular endothelium and inflammation.[123]

Inflammatory cells such as neutrophils were shown to participate in sickle cell–related vaso-occlusive events; these cells were able more efficiently to adhere to fibronectin and vascular endothelial cells than neutrophils from normal subjects. This phenomenon seems to be related to the higher surface expression of adhesion molecules used for transendothelial migration.[124,125] In fact, β2 integrin Mac 1 ($\alpha_M\beta_2$ or CD11b/CD18) was reported to be increased in neutrophils from SCD patients, suggesting a higher ability of SCD neutrophils to firmly adhere to vascular endothelium surface than normal controls with local reduction of blood flow, which is crucial in the development of acute vaso-occlusive events.[126,127] Hidalgo et al reported an interesting connection between neutrophils, red blood cells, and activated endothelial surface in developing vascular occlusion and hypoxic cell damage in microcirculation of lung from a mouse model of SCD.[128] These authors hypothesized that the interaction between these blood cell types and the activated endothelium might be coordinated by the interactions of endothelial E-selectin, neutrophil E-selectin ligand (ESL)-1, and the leukocyte integrin Mac1 ($\alpha_M\beta_2$ CD11b/CD18). The heterotypic aggregates are generated by the binding of ESL-1 on neutrophils to E-selecting on the vascular endothelium, which signals the activation of Mac 1 on neutrophils and in turn mediates the heterotypic association of neutrophils with sickle red blood cells.[128] This interesting pathway, E-selectin-ESL-Mac1, should be further studied in human subjects with SCD and might be explored as a possible target to design new pharmacological strategies in treatment sickle cell–related vaso-occlusive events.

Increased iNKT cells, nonphagocytic inflammatory cells producing proinflammatory cytokines were observed in SCD patients, and it was proposed that iNKT cells might contribute to SCD vasculopathy, representing a possible additional risk factor for stroke in sickle cell patients.[23,129]

THE NITRIC OXIDE CONNECTION

SCD is characterized by a relative reduction in NO bioavailability that contributes to abnormal endothelial activation and SCD organ damage.[14,16,85,122,130–132] NO is a potent vasodilator and inhibitor of vascular remodeling, and it also affects the multistep cascade of events involved in leukocyte, platelet, and endothelial activation. NO is generated from L-Arginine by endothelial cells via constitutive and inflammatory inducible nitric oxide synthases. Moreover, chronic hemolysis leading to increase the plasma levels of hemoglobin (i.e., an efficient

NO buffer) contributes to reduce the levels of NO in SCD. Thus the NO-reduced bioavailability might contribute to the hypercoagulable state observed in patients with SCD.

ANTIPLATELET AND ANTICOAGULANT AGENTS IN SICKLE CELL DISEASE

Information regarding the use of antiplatelet agents or anticoagulants in the treatment of either acute or chronic clinical manifestation of SCD is still limited due to the small and relative low-quality design of the studies.[17,133–138] The impact of antiplatelet agents such as aspirin or ticlopidine on either the frequency and/or duration of acute vaso-occlusive crisis were evaluated in a few studies.[17,133–138] The results were not conclusive because either no differences or limited ameliorations in the frequency and duration of acute vaso-occlusive events in SCD patients were observed.[17,133–138] Tomer et al recently reported a reduction of pain episodes and in plasma levels of F1 + 2, D-dimer, and the plasmin-antiplasmin complex in a small cohort of sickle cell patients supplemented with n-3 fatty acid, suggesting a possible correlation between a reduced prothrombotic state and the rate of acute sickle cell–related events.[29]

Anticoagulant treatment with heparin has been considered as an additional therapeutic approach to block sickle cell adhesion to endothelial cells through the P-selectin pathway[132,139–141] or binding to TSP that can mediate the interactions between sickle erythrocytes and the vascular endothelial surface. A double-blind randomized trial with tinzaparin in SCD patients during acute vaso-occlusive events documented a reduction of their severity and duration.[9,142] Studies with a low dose of warfarin or acenocoumarol were reported to slightly reduce the frequency of acute pain events with decreased thrombin generation and fibrinolysis, however without reaching a significant clinical amelioration. This therapy was also associated with frequent bleeding complications.[98,133,143,144]

Thrombolytic agents in the treatment of stroke in SCD are generally precluded due to the high risk of possible hemorrhage as a complication of thrombolytic therapy in patients presenting with moya-moya disease or pseudoxanthoma elasticum tissue abnormalities.[23,145,146]

CONCLUSION

The prothrombotic state in SCD contributes to acute and chronic clinical manifestations. Studies have shown abnormalities in the coagulation system, perturbation of platelet activation and aggregation, increased adherence of neutrophils, increased nonphagocytic iNKT cells, and abnormal red cells that more easily adhere on an abnormally activated vascular endothelial surface in SCD. Although progress has been made in characterizing the hypercoagulable state in this challenging disorder, more remains to be investigated, both related to the pathogenesis of vaso-occlusive events and the use of antiplatelet and anticoagulant treatments for a more effective clinical management of SCD patients.

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