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Role of the Hemostatic System on SCD Pathophysiology and Potential Therapeutics

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Synopsis

Recent studies suggest that sickle cell disease is a hypercoagulable state contributing to the vaso-occlusive events in microcirculation resulting in acute and chronic sickle cell related organ damage. In this article, we will review the existing evidence for contribution of hemostatic system perturbation to sickle cell disease pathophysiology. We will also review the data showing increased risk of thromboembolic events, particularly newer information on the incidence of VTE. Finally, the potential role of platelet inhibitors and anticoagulants in SCD will be briefly reviewed.

Keywords

sickle cell; thromboembolism; hypercoagulable; anti-platelets; anticoagulants

Introduction

Sickle cell disease (SCD) is the result of homozygous or compound heterozygous inheritance of mutation in the β-globin gene. The resulting substitution of the hydrophilic amino acid glutamic acid at the sixth position by the hydrophobic amino acid valine, leads to the production of hemoglobin S (HbS). HbS polymerizes when deoxygenated and this polymerization is associated with cell dehydration and increased red cell density [1-3]. The dense, rigid and sickling red cells lead to vaso-occlusion and impaired blood flow [2,4], and is thought to underlie acute (painful episodes, acute chest syndrome) and chronic (avascular necrosis, renal insufficiency) complications of the disease. Also, intracellular polymerization ultimately damages the red cell membrane and leads to chronic and episodic extra-vascular and intravascular hemolytic anemia, hemolysis linked nitric oxide (NO) dysregulation and endothelial dysfunction [5] resulting leg ulcer, pulmonary hypertension (PHTN), priapism and stroke [6].

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Several investigators have reported increased thromboembolic events and alteration in hemostatic system in SCD both under steady state and during acute events. This suggests that perturbation in hemostatic system may contribute to SCD pathophysiology. Changes that have been described include increased expression of tissue factor on blood monocytes [7-9] and endothelial cells [10,11], abnormal exposure of phosphatidylserine on the red cell surface [12,13] and increased microparticles, which both promote activation of coagulation cascade [14-16]; and high incidence of anti-phospholipid antibodies [17,18]. In fact, sickle cell disease meets the requirements of Virchow's triad (slow flow, activated pro-coagulant proteins and vascular injury); therefore, it should not be surprising that sickle disease is accompanied by thrombosis. Clinical manifestations of the prothrombotic state of sickle cell patients include venous thromboembolism (VTE), in situ thrombosis, and stroke.[2,19-22].

In this section we will highlight the existing evidence for contribution of hemostatic system perturbation to sickle cell disease pathophysiology. We will also review the data showing increased risk of thromboembolic events, particularly newer information on the incidence of VTE. Finally, the potential role of platelet inhibitors and anticoagulants in SCD will be briefly reviewed.

Evidence for Increased Thromboembolic Events in Sickle Cell Disease

Stroke has an overall prevalence of 3.75% in patients with SCD and 11% in patients under 20 years of age with sickle cell anemia (HbSS), and is most often caused by large vessel arterial obstruction with superimposed thrombosis [20,23]. New and old thrombi in the pulmonary vasculature are prevalent in autopsy series [21,24,25]. The analysis of a large discharge database in the state of Pennsylvania from 2001 to 2006 found that the incidence of pulmonary embolism was 50-fold to 100-fold higher in the SCD population (0.22–0.52%) than in the general Pennsylvania population (0.0039–0.0058%) [26]. A retrospective study of reported discharge diagnoses showed that patients with SCD younger than 40 years were more likely to be diagnosed with pulmonary embolism compared to African Americans without SCD (0.44% vs. 0.12%); however, the prevalence of deep vein thrombosis was similar between the two groups [19]. In contrast, in a retrospective study of 404 sickle cell patients cared for at the Sickle Cell Center for Adults at Johns Hopkins between August 2008 and January 2012, 25% of the patients had a history of VTE (18.8% non-catheter related), with a median age at diagnosis of 30 years. Sickle cell variant genotypes, such as HbSC or HbSβ^+ thalassemia, were associated with increased risk of non-catheter-related VTE compared to HbSS. A history of non-catheter-related VTE was an independent risk factor for death in adults with sickle cell disease [27].

SCD also appears to be a significant risk factor for pregnancy-related VTE, with an odds ratio of 6.7 [28,29]. A retrospective study showed that patients with sickle cell disease had more antenatal complications than those with sickle cell trait, without affecting the fetal outcome [30]. Sickle cell trait is generally benign, but one study suggested that sickle cell trait increases the risk of VTE in pregnancy compared to race matched controls, with an odds ratio of about 2.5. While in another study of pregnancy, sickle cell trait was associated with pulmonary embolism (PE) rather than deep vein thrombosis [31], in a recent larger study investigators could not detect a statistically significant difference in peripartum VTE or PE incidence between women with and without sickle cell trait in a large hospital cohort study [32].

Evidence of Hemostasis System Alteration in SCD

The pathophysiology of hypercoagulability in sickle cell disease is multi-factorial and is a result of alteration in almost every component of the hemostasis system (Table 1). These

alterations in platelets, pro-coagulant, anticoagulant and fibrinolytic systems are overall pro-thrombotic.

Activation of the Coagulation Cascade

Many investigators have shown biomarker evidence for ongoing activation of the coagulation cascade both during steady state (clinically well) and during vaso-occlusive crisis (VOC). These markers denote an ongoing hypercoagulable state in SCD. Thrombin generation is increased in SCD evidenced by increased prothrombin fragment 1.2 (F1.2), thrombin-antithrombin complexes, plasma fibrinogen products, D-dimer, and decreased factor V [33-35]. Also, Factor VII and activated Factor VII are decreased in SCD compared with non-SCD individuals, most likely due to accelerated FVII turnover by increased tissue factor (TF) activity [36,37]. High levels of thrombin-antithrombin complex, prothrombin fragment F1.2, and D-dimer are associated with an activated vascular endothelium in sickle cell subjects with pulmonary arterial hypertension (PAH, defined by echocardiographic criteria), and this correlates with the rate of hemolysis is these patients [38]. However, in a cohort of SCD patients with mild pulmonary hypertension, there was no association between the hypercoagulable state of SCD and the early phase of PAH [39].

Alterations in proximal intrinsic pathway proteins have been reported as well. In a small study of homozygous SS disease, plasma prekallikrein levels were decreased during steady state with a further reduction during vaso-occlusive crisis [40]. In a concomitant study, there was an additional 50% decrease in kininogen during vaso-occlusive crisis compared to low levels of kininogen at baseline. High molecular weight kinonogen (HMWK) was not directly evaluated, but plasma kallikrein almost exclusively digests HMWK therefore contact activation is the likely cause of the decrease in plasma kininogen levels [41]. The levels of Factor XII, HMWK, and prekallikrein are slightly decreased in children with homozygous SS disease in steady state [42]. Since components of the contact system are mediators of inflammation, including pain and local vasodilatation, activation of this system might play a role in inflammatory pathway perturbations as wells as coagulation pathway abnormalities that contribute to SCD pathophysiology.

Reduction in Physiological Anticoagulants' Level

Decreases in anticoagulant proteins of hemostasis system would further promote a hypercoagulable state and have been reported in SCD. Onyemlukwe et al [43] described significantly lower level of plasma anti-thrombin III (AT-III, now called antithrombin or AT) in patients with SCD compared to healthy non-SCD controls. The anticoagulants Protein C and S are low in SCD patients in steady state (crisis free), and tend to decrease to even lower levels during crisis episodes [35,44,45]. In a survey of SCD patients in steady state from Turkey, protein C and AT were significantly lower compared to non-SCD controls [46]. Also, significantly decreased levels of proteins C and S were reported in patients with SCD who developed thrombotic strokes compared with neurologically normal children with SCD [47]. However, the association with stroke was not seen in the report by Liesner and colleagues [48] despite demonstrating a reduction in protein C and S and increased thrombin generation (denoted by increased thrombin-antithrombin complexes and prothrombin fragment 1+2) in the steady state which was only partially reversed by transfusion [48]. Plasma levels of the serine protease inhibitor, heparin cofactor II (HCII), are also decreased in SCD [49]. In total these findings suggest a possible alteration in either anticoagulant synthesis related to liver disease or chronic consumption due to increased thrombin production.

Impaired Fibrinolysis

The thrombophilia in SCD is also associated with abnormalities in the fibrinolytic system, characterized by increased plasma levels of plasminogen activator inhibitor (PAI)-1 in both steady state and during sickle acute events compared to normal population [50,51]. This may be a result of increased synthesis of PAI-1 by damaged endothelial cells and activated platelets [52], and might participate in the pathogenesis of VOC in SCD. Elevations in plasma plasmin-anitplasmin complexes (PAP) [33] were also observed in patients with SCD in the non-crisis, steady state. The frequency of pain episodes in patients with SCD correlated with the extent of fibrinolytic activity (assessed by D-dimer levels) in the non-crisis steady state, suggesting that D-dimer levels may predict the frequency of pain crises [33].

Activated Platelets

Platelet abnormalities (function, number and survival) both in baseline crisis free state and acute events were some of the earliest hemostatic changes documented in sickle cell disease [53-60]. Several biomarkers have been measured to document the functional abnormalities. Urinary thromboxane- A2 and prostaglandin metabolites are increased and platelet trombospondin-1 level is decreased in sickle cell disease [61,62]. These findings suggested ongoing platelet activation. Increased platelet activation markers such as P-selectin (CD62), CD63, activated glycoprotein (GP)IIb/IIIa, plasma soluble factors (PF)-3, PF4, β-thromboglobulin and platelet-derived soluble CD40 ligand (sCD40L) were reported in SCD patients using a cytofluorimetric approaches [33,59,63,64]. Also, platelets adherence to fibrinogen was found to be increased through modulation of intracellular signaling pathways associated with increased αIIβ3-integrin activation [65]. Platelet aggregation in adults has been found to be increased, perhaps due to an increase in the number of megathrombocytes in the peripheral circulation [60,66,67] or as a result of increase in levels of platelet agonists, such as thrombin, adenosine diphosphate, or epinephrine. In contrast to adults, platelet aggregation in children was normal or reduced, perhaps due to better preservation of splenic function or fewer circulating megathrombocytes [53,55,57]. Increased phosphatidylserine rich platelets have also been described in SCD patients, which might accelerate the activation of the coagulation system [33].

Platelet number and survival are also abnormal in both steady state and acute events. In steady state there is moderate thrombocytosis in older children and adults with sickle cell anemia [66]. The number of circulating megathrombocytes, which are young and metabolically active platelet is also increased. These findings have been attributed to the functional asplenia exhibited by these patients [67]. While studies performed during steady state suggest normal platelet survival [54,68] decrease in platelet lifespan have been reported in VOC [54,69,70]. Platelet and megathrombocyte counts may decrease markedly, especially when the crisis is severe [56]. These decreases are followed by marked rebound increases in platelet and megathrombocyte counts, with levels peaking 10 to 14 days after the onset of the crisis [54,70].

All of these findings suggest that both shortened platelet survival and enhanced platelet consumption occur during vaso-occlusive crises, possibly because platelets are being deposited at sites of vascular injury or vascular occlusion. It has been demonstrated that labeled platelets accumulate at the putative sites of vaso-occlusion [69].

Pathophysiology of Hemostasis System Activation in Sickle Cell Disease

As shown above, in sickle cell disease there is a chronic increase in plasma markers of thrombin generation, decrease in natural anticoagulants and inhibited fibrinolytic system, and some data shows these changes are accentuated during a VOC. Although, the role of genetic predisposition for thrombophilia in sickle cell disease (separate from the sickle cell

mutation itself) is still under investigation, several other factors have been identified as contributors to the altered hemostatic system in SCD. While some of these play major roles in the pathophysiology of sickle cell disease such as altered RBC membrane, inflammation due to vaso-occlusion/reperfusion oxidative stress, hemolysis resulting in cell free hemoglobin, abnormal bioavailability of NO and endothelial dysfunction, the role of others remain under investigation. These include activated circulating endothelial cells, monocytes, microparticles and platelets. All of these factors have potential to activate the coagulation cascade by increased tissue factor expression on endothelial cells, monocytes, and circulating microparticles derived from RBCs, monocytes, endothelial cells and platelets [9,71]. Possible mechanisms for increased TF expression in SCD are: 1) increased cell free heme from hemolysis inducing TF expression on vascular endothelial cells [72]; 2) ischemia-reperfusion (hypoxia and inflammation). In fact in an experimental mouse model, 3 hours of exposure to a hypoxic environment and subsequent return to 18 hours of ambient air resulted in an increase in pulmonary veins tissue factor expression, suggesting that ischemia-reperfusion injury in patients with SCD may play a role in activation of pro-coagulant proteins [71]; and, 3) Platelet activation and exposure of CD40 ligand [63].

Role of Red Blood Cell Membrane

There has been extensive research on abnormal RBCs membrane and its role in pathophysiology of sickle cell disease, which is beyond the scope of this review. In summary, abnormal phosphatidylserine (PS) exposure of the sickle cell RBC membrane [12] alters the adhesive properties of sickle RBCs [73] leading to an increase in capillary transit time and stasis enhancing the potential for the activation of coagulation factors and cellular elements in the microvasculature and post-capillary venules. Additionally, the exposed PS functions as a docking site for pro-coagulant proteins [74-76]. The number of PS positive sickle RBCs significantly correlate with plasma levels of prothrombin fragment 1.2, D-dimer and PAP complexes [75,76] and may contribute to increased risk of stroke in SCD [77]. These PS positive RBCs are a signal for apoptosis [78,79] and lead to tissue factor positive microparticle generation. Antiphospholipid antibodies against PS are also markedly elevated in homozygous SCD and correlate strongly with plasma D-dimer suggesting a role for anti-phospholipid antibodies in coagulation activation in SCD [35]. The mechanism may be inhibition of protein S binding to β_2- glycoprotein-1 [80] resulting in inactivation of protein S by circulating C4b-binding protein. In addition, red blood cells with increased PS exposure may bind directly too protein S, contributing to reduction in free protein S [81,82].

Role of Hemolysis-Free Hemoglobin-NO-Spleen axis

Physiologically, endothelial-derived nitric oxide is protected from the scavenging effects of intracellular hemoglobin by the erythrocyte membrane barrier and the cell free zone that forms along endothelium in laminar flowing blood [83-86]. Also, haptoglobin, CD163, hemoxygenase and biliverdin reductase detoxify cell free hemoglobin after hemolysis. However, during intravascular hemolysis, the haptoglobin-hemoxygenase-biliverdin reductase system is overwhelmed. Consequently, the cell free hemoglobin is accumulated in plasma and interacts with nitric oxide, generating reactive oxygen species. In addition, arginase I, which is released from the red blood cell during hemolysis, metabolizes arginine which is the substrate for nitric oxide synthesis [87].

Nitric oxide not only regulates the vascular tone and inhibits endothelial adhesion molecule expression, but also has potent antithrombotic effects. Nitric oxide inhibits platelet activation via cGMP-dependent signaling [88-91]. Nitric oxide may also inhibit tissue factor expression [92-94].

In addition to scavenging NO, cell free hemoglobin, can inhibit ADAMTS13 activity affecting VWF cleavage in patients with TTP [95]. In fact, ADAMTS13 activity and ADAMTS13 to VWF antigen ration is decreased in sickle cell patients compared to normal controls [96-98]. This would block the proteolysis of vWF and could lead to the accumulation of ultra large adhesive VWF on the vascular endothelium surface and thrombosis. This mechanism may be a novel factor contributing to the complex microvascular pathophysiology of SCD. As mentioned earlier, free heme can also induce the endothelial tissue factor expression.

The spleen clears senescent, oxidized and phosphatidylserine-exposing red cells from the circulation and thus limits intravascular cell microvesiculation, hemolysis and phosphatidylserine exposure [99-101]. Increase in the plasma concentration of cell free hemoglobin and red cell microparticles after splenectomy could increase nitric oxide scavenging, vascular injury and thrombosis. Interestingly, since priapism may also be a complication of hemolytic anemia and low nitric oxide bioavailability [102-104], an increase in intravascular cell free plasma hemoglobin and red cell microparticles after splenectomy could also explain the observed development of priapism after splenectomy [105,106]. It is also well established that splenectomy in other patient populations is associated with increased risk of VTE [107].

The Microparticles

Microparticles are small membrane vesicles released from cells when activated or during apoptosis. Microparticles (MP) in the blood can originate from platelets, erythrocytes, leukocytes and endothelial cells [108]. In healthy individuals, circulating MPs are mainly derived from platelets and to a lesser extent leukocyte and endothelial cells [109]. Elevated numbers of circulating microparticles have been reported in patients suffering from a variety of diseases with vascular involvement and hypercoagulability, including SCD [110-115]. The exact mechanism by which circulating microparticles trigger coagulation in SCD remains unclear. The majority of circulating microparticles in SCD originate from erythrocytes and platelets and by exposure of phosphatidylserine facilitate coagulation cascade complex formation.

Additionally, increased exposure of tissue factor has been demonstrated on monocyte-derived microparticles [110]. Tissue factor (TF) positive microparticles derived from red blood cells, platelets, endothelial cells and monocytes are elevated in sickle cell patients both in steady state and during acute events compared to normal controls [110] suggesting their possible role in sickle cell prothrombotic state [48]. The high levels of erythroid and platelet derived microparticles in sickle cell patients may further increase during acute vaso-occlusive events, although this has not been a consistent finding [59].

The erythroid derived microparticles are able to activate the coagulation system independent of the tissue factor and their level correlates with markers of hemolysis, von Willebrand factor, D-dimer and F1+2 levels [14], pain crisis and elevated tricuspid regurgitant jet velocity measured by echocardiogram [15]. Their ability to activate the coagulation system through factor XIIa [16] could explain this third pathway. The increase in thrombin generation seems to primarily be caused by erythroid derived microparticles and hydroxyurea is associated with decreased circulating microparticles compared to untreated patients [116].

Genetic predisposition for Thrombophilia in Sickle Cell Disease

Genetic modifiers with functional effects on hemostatic system have been studied in patients with sickle cell disease. Many of the thrombophilic mutations described to date are not prevalent in people of African descent [117,118]. However, in some populations, sickle cell

patients might be carrying thrombophilic mutations more than general population. Studies of Human Platelet Alloantigen (HPA) polymorphism showed a possible pro-thrombotic role in these patients. Few investigators have studied role of genetic modifiers of vascular endothelium as well.

Thrombophilic Mutations

Many studies reported the low frequency of thrombophilic mutations (Factor V Leiden, MTHFR C677T, and prothrombin G20210A) and the lack of association between these mutations and risk of thromboembolism in African American, sub-Saharan African, West Indies, Maghrib, Brazilian, Jamaican and eastern Saudi Arabian SCD patients, possibly due to low frequency of these genes in the related general population [119-127]. However, despite the moderate prevalence of Factor V Leiden (FVL) mutation (2.97-5.5%) among the general population of Iran [128], the prevalence of FVL mutation is higher (14.3%) among Iranian SCD patients [129] with a significant association between this mutation and SCD (OR=6.5). Also, a study from Brazil suggests that MTHFR C677T might be a risk factor for vascular complications in SCD [123]. Also, three studies of inherited risk factors of venous thromboembolism in SCD patients from Southern Mediterranean countries [130-132], report high prevalence of thrombophilic mutations in SCD patients and their association with thromboembolic events. Among Lebanese sickle/β^0-thalassemia patients, a high prevalence of the thrombophilic mutations of FVL (42%), homozygous and heterozygous MTHFR C677T (59%), and prothrombin G20210A (8%) has been reported [130]. In this report sickle/β-thalassemia patients were 5.24 and 4.39 fold more likely to have FVL mutation as compared to the normal controls and thalassemia intermedia patients, respectively. Also, the presence of extensive large vessel thrombosis in a sickle/β^0-thalassemia patient from Lebanon who was homozygous for FVL and heterozygous for MTHFRC677T has been reported [131]. In another case report, a sickle cell patient from Israel with recurrent cerebrovascular accident and deep venous thrombosis, was found to be heterozygous for FVL and MTHFR C677T [132]. Therefore, the prevalence of thrombophilic mutations in non-African SCD population may be of clinical relevance.

Human Platelet Alloantigen Polymorphism

Polymorphisms in human platelet alloantigen genes may determine platelet reactivity and have been associated with variable risk of thrombotic events, mostly arterial [133]. Studies on polymorphisms of human platelet alloantigen (HPA), show a possible pro-thrombotic role in different thrombotic disorders and in sickle cell patients with cerebrovascular events [133-137]. 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, is an independent risk factor for acute vaso-occlusive events in SCD [137].

Genetic modifiers affecting vascular endothelium in patients with sickle cell disease have been evaluated in a few studies, however the mechanism by which they affect the vascular endothelium needs to be investigated further and in larger patient populations.

Therapeutic Implications of Hemostatic System Activation in Sickle Cell Disease

Although hemostatic activation is somewhat downstream in the SCD pathophysiological cascade, it is plausible that a therapy targeted at decreasing platelet and coagulation activation might ameliorate or prevent sickle cell related complications. This is analogous to the use of platelet inhibitors in atherosclerotic vascular disease and anticoagulants in venous thromboembolism. The underlying pathogenesis is not targeted; nonetheless, blocking downstream effects does decrease the incidence and severity of complications. In addition,

emerging data suggesting increased venous thromboembolism in patients with sickle cell disease [26,27,138], provides further rationale for treatment with either platelet inhibitors or anticoagulants. Finally, platelet inhibitors and anticoagulants are widely used and studied, and their safety profiles well known in diverse populations. All of this makes study of these agents attractive in sickle cell disease.

Trials of Platelet Inhibitors in Sickle Cell Disease

To date there are only three studies in humans evaluating the therapeutic effect of *aspirin* in sickle cell disease (Table II). These studies were conducted in 1980s and results are limited by the study design. When hemoglobin was incubated with aspirin in-vitro, the acetyl group was incorporated into hemoglobin and lead to increased oxygen affinity [139]. Subsequently, Osamo et al. investigated the therapeutic effect of aspirin in 100 patients with homozygous SCD aged 11-20 years [140]. In this study half of patients were randomized to receive total daily dose of 1200 mg soluble aspirin for 6 weeks while the other half received placebo in addition to usual care. Hemoglobin levels and oxygen saturation increased in the aspirin arm with increased red cell survival in the 3 patients whose red cell survival was measured. There were no comparative values for placebo arm. There were no serious hemorrhagic events in the treatment group. Pain was not formally assessed. However, in a double-blind placebo-controlled cross-over study of a lower dose of aspirin (3-6 mg/kg) for longer period of time (21 months) in 49 children with HbSS, HbSC, or HbSO-Arab aged 2-17 years, there was no difference in the number of painful episodes, number of total days in pain, duration of pain crisis, or pain severity during crisis between the aspirin and placebo treated periods using pain assessment forms completed by their parents. Irrespective of the treatment, there was a marked decrease in the number of pain crises after the first 6 months of study [141]. Similarly, a single-blind crossover study of 29 patients age 4-31 years receiving 17-45 mg/kg/day of aspirin for five months followed by no aspirin for the next five months [142], did not find difference in the painful events.

The data for use of *dipyridamole* in sickle cell disease is sparse. Chaplin and colleagues treated 3 patients with aspirin 650 mg PO twice a day and dipyridamole (a phosphodiesterase inhibitor) 50 mg PO twice daily for acute pain crisis and compared the frequency and severity of pain for the 2 years on therapy to the next 2 years off the therapy [143]. The severity of pain and the total number of hospitalizations for pain decreased. During the study there was no evidence of increased bleeding.

Therapeutic effect of *thienopyridines* has also been studied in sickle cell disease. Semple and colleagues assessed platelet survival and activation in 9 asymptomatic patients with SCD [69] who were randomized to placebo for 28 days followed by *ticlopidine* 250 mg PO twice daily for next 28 days. Ticlopidine did not prolong platelet survival (measured by radio-labeled platelets) but 40% reduction in collagen and ADP-induced maximal platelet aggregation was observed in this double-blind placebo-controlled trial. One patient had a painful episode during the therapy, but this study was not powered to determine a difference in pain. Cabannes et al. randomized 140 SCD patients to ticlopidine 500 - 750 mg daily for 6 months or placebo to study the efficacy of ticlopidine in the prevention of acute pain crisis [144]. Frequency of crisis, crisis duration, and crisis severity decreased in the ticlopidine arm compared with the placebo arm. More recently, Wun et al [138] studied the third generation thienopyridine, *prasugrel*, in a randomized, double blind adaptive Phase 2 study in adults with all genotypes of sickle cell disease. Patients were randomized to prasugrel 5 mg daily (n=41) or placebo (n=21) for 30 days. Platelet function was significantly inhibited in prasugrel compared with placebo treated SCD patients. Biomarkers of in vivo platelet activation, including platelet surface P-selectin and plasma soluble P-selectin, were significantly reduced in SCD patients treated with prasugrel compared with placebo. Mean

pain rate (percentage of days with pain) and intensity decreased in the prasugrel arm but did not reach statistical significance. Prasugrel was well tolerated and not associated with serious hemorrhagic events. Despite the small size and short duration of this study, there was a decrease in platelet activation biomarkers and a trend toward decreased pain. An international Phase 3 study of prasugrel in children with sickle cell disease is currently enrolling.

Anticoagulant therapy for Sickle Cell Disease

Anticoagulants might be used in SCD for the primary or secondary prevention of VTE, to treat or prevent complications of SCD, or both. For the primary or secondary prevention of VTE, there is no evidence that the use of anticoagulant medications should be any different than in other medically ill patients with regard to indications, dose, intensity, or duration of therapy. This is due to the lack of SCD specific studies addressing the management of VTE in this population. Special considerations include the possibility that filling defects on CT angiogram may represent in situ sickling rather than a classic fibrin-rich clot, and the lack of utility of D-dimer in diagnostic algorithms for VTE due to persistent elevations in many patients. In addition, if one considers SCD to be a potent and persistent hypercoagulable state, then there is a stronger argument for extended secondary prophylaxis after a first unprovoked event. This would be concordant with current American College of Chest Physician guidelines that suggest extended anticoagulation after unprovoked events for all patients when there is low risk of bleeding.

There have been a handful of studies examining chronic anticoagulation in SCD; most have been small and uncontrolled (Table III). A series of 12 patients treated with *warfarin* was reported by Salvaggio and colleagues in 1963 [145]. Each patient served as his/her own control. Although no formal statistics were performed, the frequency of pain episodes did not seem to improve and there were 7 episodes of bleeding, 1 nearly fatal. The authors concluded that warfarin was not beneficial in SCD.

Schnog and colleagues [146] performed a randomized, double blind, placebo-controlled, cross-over pilot study to assess the efficacy and safety of low-adjusted dose *acenocoumarol*. Treatment was either acenocoumarol or placebo for 14 weeks, after which treatment was discontinued for a period of five weeks. Patients were then crossed over. Efficacy was assessed by comparing the frequency of VOC, incident bleeding, and biomarkers of coagulation activation between acenocoumarol and placebo treatment of each patient. Twenty-two patients (14 homozygous [HbSS] and 8 compound heterozygous sickle-C [HbSC]; aged 20-59 years) completed the entire study. Acenocoumarol treatment did not result in a significant reduction of VOC events. There was a marked reduction of the hypercoagulable state as denoted by biomarkers (decreased plasma levels of prothrombin F1.2 fragments [P = 0.002], thrombin-antithrombin complexes [P = 0.003], and D-dimer fragments [P = 0.001]) without the major bleeding. Even though no clinical benefit (pertaining to the frequency of painful crises) was detected in this pilot study, the authors concluded the value of low adjusted-dose acenocoumarol for preventing specific events (such as strokes) and as a long-term treatment of sickle cell patients should be subject of further study.

Anticoagulation has also been studied in the acute VOC setting. Ahmed and colleagues [147] measured plasma D-dimer in 37 adult patients with SCD who were hospitalized for VOC. D-dimer level of patients who were on low-dose *warfarin* was compared with those patients who were not on any anticoagulation treatment. Patients were on warfarin either for VTE or catheter prophylaxis; this was not a randomized study. Overall median D-dimer level in 65 samples was 2.7 microgram fibrinogen equivalent units (FEU)/mL (0.34-4). Patients who were on low-dose warfarin had a median D-dimer level of 0.81 microgram FEU/mL

(0.34-1.8) compared with 3.1 microgram FEU/mL (0.94-4) in those patients who were not on anticoagulation treatment. Using ANOVA to model D-dimer levels, only warfarin was significantly correlated with low D-dimer levels after controlling for other variables. They concluded that patients with SCD during vaso-occulssive painful crisis have an elevated D-dimer level and that low-dose anticoagulation treatment is associated with a significant reduction in the D-dimer levels. There was no assessment of clinically important outcomes.

A randomized double-blind clinical trial was performed to test the safety and efficacy of a low-molecular-weight heparin, *tinzaparin*, for the management of VOC [148]. Two hundred fifty-three patients with acute painful crisis but with no other complications of SCD, were randomized to tinzaparin at 175 IU/kg, subcutaneous once daily, along with supportive care including morphine analgesia; in the comparator group, 126 patients received placebo and the same supportive care. The maximal treatment period was seven days. Tinzaparin-treated patients had significantly fewer total hospital days (mean of 7.08 vs. 12.06 days), crisis duration (mean of 2.57 vs. 4.35 days) and days of severest pain score (mean of 1.28 vs. 1.74) compared to placebo-treated patients. There were two minor bleeding events in the tinzaparin arm. As noted, heparin in the setting of SCD may inhibit P-selectin mediated red cell adhesion in addition to anticoagulant effects. These provocative findings should be confirmed in future studies, especially as one could argue low molecular weight heparin at prophylactic doses should be routine is hospitalized sickle cell patients.

Summary

Although the pathogenesis of sickle cell disease lies in disordered hemoglobin structure and function, downstream effects of sickle hemoglobin include changes in hemostatic system that overall result in a pro-thrombotic phenotype. These changes include thrombin activation, decreased levels of anticoagulants, impaired fibrinolysis and platelet activation. Limited studies to date suggest that biomarkers of activation can be affected by currently available anti-thrombotic drugs, and provocative data from pilot studies indicate there may be improvement in clinically important outcomes. Therefore, clinical trials with antithrombotic therapies, are justified with both SCD related complications (VOC, pain) and thrombotic complications as outcome events of interest.

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- Although the pathogenesis of sickle cell disease lies in disordered hemoglobin structure and function, downstream effects of sickle hemoglobin include changes in hemostatic system that overall result in a pro-thrombotic phenotype.
- These changes include thrombin activation, decreased levels of anticoagulants, impaired fibrinolysis and platelet activation.
- Limited studies to date suggest that biomarkers of activation can be affected by currently available anti-thrombotic drugs, and provocative data from pilot studies indicate there may be improvement in clinically important outcomes.
- Therefore, clinical trials with antithrombotic therapies, are justified with both SCD related complications (VOC, pain) and thrombotic complications as outcome events of interest.

Table I

Hemostatic alterations in patients with sickle cell disease

Increased Levels	Decreased Levels
Platelet activation	Factor V
Platelet Aggregation	Factor XII
Phosphatidylserine-rich platelets	Factor IX
Thrombin-antithrombin complexes	Protein C
Prothrombin fragment F 1.2	Protein S
Plasmin-antiplasmin complexes	
Fibrinogen and fibrin-fibrinogen complex	
Fibrinopeptide A	
D-dimer	
Plasminogen activator inhibitor (PAI)	

Adapted from De Franceschi et al[149]

Table II

Studies of Platelet Inhibition in Sickle Cell Disease

Author	Genotypes	Study Type (N)	Therapy	Overall Result
Chaplin et al[143]	HbSS	Non-randomized cross-over (3)	Aspirin and Diypyridamole	Decrease in pain frequency, platelet count and fibrinogen
Osamo et al[140]	HbSS	Randomized (100)	Aspirin	Increase in oxygen affinity, Hb, and red cell life span Pain not formally assessed
Greenberg et al[141]	HbSS/SO^{Arab}/SC	Randomized (49)	Aspirin vs. placebo	No decrease in pain frequency
Semple et al[69]	HbSS/Sβ thalassemia	Randomized (9)	Ticlopidine vs. placebo	No change in pain, but decrease in platelet activation biomarkers
Cabannes et al[144]	HbSS	Randomized (140)	Ticlopidine vs. placebo	Reduction in frequency and duration of VOC
Zago et al[142]	HbSS/Sβ thalassemia	Randomized (29)	Aspirin vs. Placebo	No change in pain episodes or laboratory values
Wun et al[138]	HbSS/Sβ thalassemia/SC	Randomized Phase 2 (62)	Prasugrel vs. Placebo	Decrease in platelet activation and trend to decreased pain frequency and rate

Adapted from Ataga and Key[150]

Table III

Studies of anticoagulation in sickle cell disease

Author	Genotypes	Study Type (N)	Therapy	Overall results
Salvaggio et al[145]	HbSS	Non-randomized (12)	Warfarin	Slight decrease in frequency of pain episodes
Chaplin et al[143]	HbSS	Non-randomized (4)	Heparin	Reduced frequency of pain episodes
Wolters et al1[151]	HbSS/SC	Non-randomized (7)	Acenocoumarol	Reduction in biomarker of thrombin activation
Schnog et al[146]	HbSS/SC	Randomized (22)	Acenocoumarol vs. placebo	Decreased markers of thrombin activation but no effect on pain
Qari et al[148]	HbSS	Randomized (253)	Tinzaprain vs. placebo	Reduction in the duration and severity of VOC

Adapted from Ataga and Key[150]