Published in final edited form as:

Hematol Oncol Clin North Am. 2014 April ; 28(2): 355–374. doi:10.1016/j.hoc.2013.11.011.

Role of the Hemostatic System on SCD Pathophysiology and Potential Therapeutics

Zahra Pakbaz, MD¹ and Ted Wun, MD^{1,2,3}

¹Division of Hematology Oncology, University of California, Davis School of Medicine, Sacramento, CA

²UC Davis Clinical and Translational Sciences Center, Sacramento, CA

³VA Northern California Health Care System, Sacramento, CA

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 density¹⁻³. 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 dysfunction⁵ resulting leg ulcer, pulmonary hypertension (PHTN), priapism and stroke⁶.

Corresponding author: Ted Wun, MD.

Authors contact information

Address/Phone/Fax (for both authors): University of California, Davis Cancer Center, 4501 X St., Ste. 3016, Sacramento, CA 95817, **Phone:** (916) 734-3772, **Fax:** (916) 734-7946, zahra.pakbaz@ucdmc.ucdavis.edu, ted.wun@ucdmc.ucdavis.edu

ZP has no conflict of interest.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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 monocytes⁷⁻⁹ 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 cascade¹⁴⁻¹⁶; 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 groups¹⁹. 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 disease²⁷.

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 outcome³⁰. 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 thrombosis³¹, 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 study³².

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 V³³⁻³⁵. 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 in these patients³⁸. 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 PAH³⁹.

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 crisis⁴⁰. 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 kininogen (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 levels⁴¹. The levels of Factor XII, HMWK, and prekallikrein are slightly decreased in children with homozygous SS disease in steady state⁴². 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 well 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 al⁴³ 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 controls⁴⁶. 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 SCD⁴⁷. However, the association with stroke was not seen in the report by Liesner and colleagues⁴⁸ 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 transfusion⁴⁸. Plasma levels of the serine protease inhibitor, heparin cofactor II (HCII), are also decreased in SCD⁴⁹. 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 platelets⁵², and might participate in the pathogenesis of VOC in SCD. Elevations in plasma plasmin-antiplasmin 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 crises³³.

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 disease⁵³⁻⁶⁰. 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 α IIb β 3-integrin activation⁶⁵. 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 system³³.

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 anemia⁶⁶. 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 patients⁶⁷. 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 severe⁵⁶. 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-occlusion⁶⁹.

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 cells⁷²; 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 proteins⁷¹; and, 3) Platelet activation and exposure of CD40 ligand⁶³.

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 membrane¹² alters the adhesive properties of sickle RBCs⁷³ 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 proteins⁷⁴⁻⁷⁶. 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 SCD⁷⁷. 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 SCD³⁵. The mechanism may be inhibition of protein S binding to β_2 -glycoprotein-1⁸⁰ resulting in inactivation of protein S by circulating C4b-binding protein. In addition, red blood cells with increased PS exposure may bind directly to 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 blood⁸³⁻⁸⁶. 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 synthesis⁸⁷.

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 signaling⁸⁸⁻⁹¹. Nitric oxide may also inhibit tissue factor expression⁹²⁻⁹⁴.

In addition to scavenging NO, cell free hemoglobin, can inhibit ADAMTS13 activity affecting VWF cleavage in patients with TTP⁹⁵. In fact, ADAMTS13 activity and ADAMTS13 to VWF antigen ratio is decreased in sickle cell patients compared to normal controls⁹⁶⁻⁹⁸. 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 exposure⁹⁹⁻¹⁰¹. 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 bioavailability¹⁰²⁻¹⁰⁴, 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 VTE¹⁰⁷.

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 cells¹⁰⁸. In healthy individuals, circulating MPs are mainly derived from platelets and to a lesser extent leukocyte and endothelial cells¹⁰⁹. Elevated numbers of circulating microparticles have been reported in patients suffering from a variety of diseases with vascular involvement and hypercoagulability, including SCD¹¹⁰⁻¹¹⁵. 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 microparticles¹¹⁰. 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 controls¹¹⁰ suggesting their possible role in sickle cell prothrombotic state⁴⁸. 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 finding⁵⁹.

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 levels¹⁴, pain crisis and elevated tricuspid regurgitant jet velocity measured by echocardiogram¹⁵. Their ability to activate the coagulation system through factor XIIa¹⁶ 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 patients¹¹⁶.

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 population¹¹⁹⁻¹²⁷. However, despite the moderate prevalence of Factor V Leiden (FVL) mutation (2.97-5.5%) among the general population of Iran¹²⁸, the prevalence of FVL mutation is higher (14.3%) among Iranian SCD patients¹²⁹ 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 SCD¹²³. Also, three studies of inherited risk factors of venous thromboembolism in SCD patients from Southern Mediterranean countries¹³⁰⁻¹³², 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 reported¹³⁰. 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 MTHFR C677T has been reported¹³¹. 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 C677T¹³². 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 arterial¹³³. 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 events¹³³⁻¹³⁷. 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 SCD¹³⁷.

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 1963¹⁴⁵. 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 colleagues¹⁴⁶ 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 colleagues¹⁴⁷ 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-occlusive 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 VOC¹⁴⁸. 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 in 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.

Acknowledgments

TW receives grant funding from Glycomimetics, Inc.

References

1. Eaton WA, Hofrichter J. Sickle cell hemoglobin polymerization. *Advances in protein chemistry*. 1990; 40:63–279. [PubMed: 2195851]
2. Steinberg MH. Management of sickle cell disease. *The New England journal of medicine*. Apr 1; 1999 340(13):1021–1030. [PubMed: 10099145]
3. Ballas SK, Smith ED. Red blood cell changes during the evolution of the sickle cell painful crisis. *Blood*. Apr 15; 1992 79(8):2154–2163. [PubMed: 1562742]
4. Solovey AA, Solovey AN, Harkness J, Hebbel RP. Modulation of endothelial cell activation in sickle cell disease: a pilot study. *Blood*. Apr 1; 2001 97(7):1937–1941. [PubMed: 11264155]
5. Kato GJ, Gladwin MT, Steinberg MH. Deconstructing sickle cell disease: reappraisal of the role of hemolysis in the development of clinical subphenotypes. *Blood reviews*. Jan; 2007 21(1):37–47. [PubMed: 17084951]
6. Ohene-Frempong, K.; S, MH. Clinical Aspects of Sickle Cell Anemia in Adults and Children. In: Steinberg, BF.; BG; Higgs, DR.; Nagel, RL., editors. *Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management*. Cambridge: Cambridge University Press; 2001. p. 611-670.

7. Belcher JD, Marker PH, Weber JP, Hebbel RP, Vercellotti GM. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. *Blood*. Oct 1; 2000 96(7):2451–2459. [PubMed: 11001897]
8. Setty BN, Key NS, Rao AK, et al. Tissue factor-positive monocytes in children with sickle cell disease: correlation with biomarkers of haemolysis. *British journal of haematology*. May; 2012 157(3):370–380. [PubMed: 22360627]
9. Key NS, Slungaard A, Dandele L, et al. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. *Blood*. Jun 1; 1998 91(11):4216–4223. [PubMed: 9596669]
10. Solovey A, Gui L, Key NS, Hebbel RP. Tissue factor expression by endothelial cells in sickle cell anemia. *The Journal of clinical investigation*. May 1; 1998 101(9):1899–1904. [PubMed: 9576754]
11. Setty BN, Betal SG, Zhang J, Stuart MJ. Heme induces endothelial tissue factor expression: potential role in hemostatic activation in patients with hemolytic anemia. *Journal of thrombosis and haemostasis : JTH*. Dec; 2008 6(12):2202–2209. [PubMed: 18983524]
12. Kuypers FA, Lewis RA, Hua M, et al. Detection of altered membrane phospholipid asymmetry in subpopulations of human red blood cells using fluorescently labeled annexin V. *Blood*. Feb 1; 1996 87(3):1179–1187. [PubMed: 8562945]
13. Wood BL, Gibson DF, Tait JF. Increased erythrocyte phosphatidylserine exposure in sickle cell disease: flow-cytometric measurement and clinical associations. *Blood*. Sep 1; 1996 88(5):1873–1880. [PubMed: 8781447]
14. van Beers EJ, Schaap MC, Berckmans RJ, et al. Circulating erythrocyte-derived microparticles are associated with coagulation activation in sickle cell disease. *Haematologica*. Nov; 2009 94(11):1513–1519. [PubMed: 19815831]
15. Tantawy AA, Adly AA, Ismail EA, Habeeb NM, Farouk A. Circulating platelet and erythrocyte microparticles in young children and adolescents with sickle cell disease: Relation to cardiovascular complications. *Platelets*. Dec 18; 2012
16. Van Der Meijden PE, Van Schilfgaarde M, Van Oerle R, Renne T, ten Cate H, Spronk HM. Platelet- and erythrocyte-derived microparticles trigger thrombin generation via factor XIIa. *Journal of thrombosis and haemostasis : JTH*. Jul; 2012 10(7):1355–1362. [PubMed: 22537188]
17. Westerman MP, Unger L, Kucuk O, Quinn P, Lis LJ. Phase changes in membrane lipids in sickle red cell shed-vesicles and sickle red cells. *American journal of hematology*. Jul; 1998 58(3):177–182. [PubMed: 9662267]
18. Kucuk O, Gilman-Sachs A, Beaman K, Lis LJ, Westerman MP. Antiphospholipid antibodies in sickle cell disease. *American journal of hematology*. Apr; 1993 42(4):380–383. [PubMed: 8493989]
19. Stein PD, Beemath A, Meyers FA, Skaf E, Olson RE. Deep venous thrombosis and pulmonary embolism in hospitalized patients with sickle cell disease. *The American journal of medicine*. Oct; 2006 119(10):897, e897–811. [PubMed: 17000225]
20. Prengler M, Pavlakis SG, Prohovnik I, Adams RJ. Sickle cell disease: the neurological complications. *Annals of neurology*. May; 2002 51(5):543–552. [PubMed: 12112099]
21. Adedeji MO, Cespedes J, Allen K, Subramony C, Hughson MD. Pulmonary thrombotic arteriopathy in patients with sickle cell disease. *Archives of pathology & laboratory medicine*. Nov; 2001 125(11):1436–1441. [PubMed: 11697998]
22. Haque AK, Gokhale S, Rampy BA, Adegboyega P, Duarte A, Saldana MJ. Pulmonary hypertension in sickle cell hemoglobinopathy: a clinicopathologic study of 20 cases. *Human pathology*. Oct; 2002 33(10):1037–1043. [PubMed: 12395378]
23. Ohene-Frempong K, Weiner SJ, Sleeper LA, et al. Cerebrovascular accidents in sickle cell disease: rates and risk factors. *Blood*. Jan 1; 1998 91(1):288–294. [PubMed: 9414296]
24. Graham JK, Mosunjac M, Hanzlick RL, Mosunjac M. Sickle cell lung disease and sudden death: a retrospective/prospective study of 21 autopsy cases and literature review. *The American journal of forensic medicine and pathology*. Jun; 2007 28(2):168–172. [PubMed: 17525572]
25. Mancini EA, Culbertson DE, Yang YM, et al. Causes of death in sickle cell disease: an autopsy study. *British journal of haematology*. Oct; 2003 123(2):359–365. [PubMed: 14531921]

26. Novelli EM, Huynh C, Gladwin MT, Moore CG, Ragni MV. Pulmonary embolism in sickle cell disease: a case-control study. *Journal of thrombosis and haemostasis : JTH.* May; 2012 10(5):760–766. [PubMed: 22417249]
27. Naik RP, Streiff MB, Haywood C Jr, Nelson JA, Lanzkron S. Venous thromboembolism in adults with sickle cell disease: a serious and under-recognized complication. *The American journal of medicine.* May; 2013 126(5):443–449. [PubMed: 23582935]
28. Villers MS, Jamison MG, De Castro LM, James AH. Morbidity associated with sickle cell disease in pregnancy. *American journal of obstetrics and gynecology.* Aug; 2008 199(2):125 e121–125. [PubMed: 18533123]
29. James AH, Jamison MG, Brancazio LR, Myers ER. Venous thromboembolism during pregnancy and the postpartum period: incidence, risk factors, and mortality. *American journal of obstetrics and gynecology.* May; 2006 194(5):1311–1315. [PubMed: 16647915]
30. Zia S, Rafique M. Comparison of pregnancy outcomes in women with sickle cell disease and trait. *JPMa The Journal of the Pakistan Medical Association.* Jun; 2013 63(6):743–746. [PubMed: 23901677]
31. Austin H, Key NS, Benson JM, et al. Sickle cell trait and the risk of venous thromboembolism among blacks. *Blood.* Aug 1; 2007 110(3):908–912. [PubMed: 17409269]
32. Pintova S, Cohen HW, Billett HH. Sickle cell trait: is there an increased VTE risk in pregnancy and the postpartum? *PloS one.* 2013; 8(5):e64141. [PubMed: 23717554]
33. Tomer A, Harker LA, Kasey S, Eckman JR. Thrombogenesis in sickle cell disease. *The Journal of laboratory and clinical medicine.* Jun; 2001 137(6):398–407. [PubMed: 11385360]
34. Peters M, Plaat BE, ten Cate H, Wolters HJ, Weening RS, Brandjes DP. Enhanced thrombin generation in children with sickle cell disease. *Thrombosis and haemostasis.* Feb; 1994 71(2):169–172. [PubMed: 8191393]
35. Westerman MP, Green D, Gilman-Sachs A, et al. Antiphospholipid antibodies, proteins C and S, and coagulation changes in sickle cell disease. *The Journal of laboratory and clinical medicine.* Oct; 1999 134(4):352–362. [PubMed: 10521081]
36. Kurantsin-Mills J, Ofosu FA, Safa TK, Siegel RS, Lessin LS. Plasma factor VII and thrombin-antithrombin III levels indicate increased tissue factor activity in sickle cell patients. *British journal of haematology.* Aug; 1992 81(4):539–544. [PubMed: 1390242]
37. Hagger D, Wolff S, Owen J, Samson D. Changes in coagulation and fibrinolysis in patients with sickle cell disease compared with healthy black controls. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis.* Apr; 1995 6(2):93–99. [PubMed: 7605884]
38. Ataga KI, Moore CG, Hillery CA, et al. Coagulation activation and inflammation in sickle cell disease-associated pulmonary hypertension. *Haematologica.* Jan; 2008 93(1):20–26. [PubMed: 18166781]
39. van Beers EJ, Spronk HM, Ten Cate H, et al. No association of the hypercoagulable state with sickle cell disease related pulmonary hypertension. *Haematologica.* May; 2008 93(5):e42–44. [PubMed: 18450728]
40. Miller R, Verma P, Adams R. Studies of the kallikrein-kinin system in patients with sickle cell disease. *J Nat Med Assoc.* 1983(75):551–556.
41. Verma P, Adams R, Miller R. Reduced plasma kininogen concentration during sickle cell crisis. *Res Comm Chem Path Pharm.* 1983(41):313–322.
42. Gordon E, Klein B, Berman B, Strandjord S, Simon J, Coccia P. Reduction of contact factors in sickle cell disease. *J Pediatrics.* 1985(106):427–430.
43. Onyemelukwe GC, Jibril HB. Anti-thrombin III deficiency in Nigerian children with sickle cell disease. Possible role in the cerebral syndrome. *Tropical and geographical medicine.* Jan; 1992 44(1-2):37–41. [PubMed: 1496720]
44. Wright JG, Malia R, Cooper P, Thomas P, Preston FE, Serjeant GR. Protein C and protein S in homozygous sickle cell disease: does hepatic dysfunction contribute to low levels? *British journal of haematology.* Sep; 1997 98(3):627–631. [PubMed: 9332318]
45. el-Hazmi MA, Warsy AS, Bahakim H. Blood proteins C and S in sickle cell disease. *Acta haematologica.* 1993; 90(3):114–119. [PubMed: 8291368]

46. Bayazit AK, Kilinc Y. Natural coagulation inhibitors (protein C, protein S, antithrombin) in patients with sickle cell anemia in a steady state. *Pediatrics international : official journal of the Japan Pediatric Society*. Dec; 2001 43(6):592–596. [PubMed: 11737735]
47. Tam DA. Protein C and protein S activity in sickle cell disease and stroke. *Journal of child neurology*. Jan; 1997 12(1):19–21. [PubMed: 9010791]
48. Liesner R, Mackie I, Cookson J, et al. Prothrombotic changes in children with sickle cell disease: relationships to cerebrovascular disease and transfusion. *British journal of haematology*. Dec; 1998 103(4):1037–1044. [PubMed: 9886316]
49. Porter JB, Young L, Mackie IJ, Marshall L, Machin SJ. Sickle cell disorders and chronic intravascular haemolysis are associated with low plasma heparin cofactor II. *British journal of haematology*. Mar; 1993 83(3):459–465. [PubMed: 8485052]
50. Nsiri B, Gritli N, Mazigh C, Ghazouani E, Fattoum S, Machghoul S. Fibrinolytic response to venous occlusion in patients with homozygous sickle cell disease. *Hematology and cell therapy*. Oct; 1997 39(5):229–232. [PubMed: 9395896]
51. Nsiri B, Gritli N, Bayoudh F, Messaoud T, Fattoum S, Machghoul S. Abnormalities of coagulation and fibrinolysis in homozygous sickle cell disease. *Hematology and cell therapy*. Jul; 1996 38(3): 279–284. [PubMed: 8974793]
52. Yamamoto K, Saito H. A pathological role of increased expression of plasminogen activator inhibitor-1 in human or animal disorders. *International journal of hematology*. Dec; 1998 68(4): 371–385. [PubMed: 9885437]
53. Stuart MJ, Stockman JA, Oski FA. Abnormalities of platelet aggregation in the vaso-occlusive crisis of sickle-cell anemia. *The Journal of pediatrics*. Nov; 1974 85(5):629–632. [PubMed: 4423856]
54. Haut MJ, Cowan DH, Harris JW. Platelet function and survival in sickle cell disease. *The Journal of laboratory and clinical medicine*. Jul; 1973 82(1):44–53. [PubMed: 4718003]
55. Gruppo RA, Glueck HI, Granger SM, Miller MA. Platelet function in sickle cell anemia. *Thrombosis research*. Mar; 1977 10(3):235–235. [PubMed: 854872]
56. Freedman ML, Karparkin S. Elevated platelet count and megathrombocyte number in sickle cell anemia. *Blood*. Oct; 1975 46(4):579–582. [PubMed: 1174691]
57. Mehta P, Mehta J. Abnormalities of platelet aggregation in sickle cell disease. *The Journal of pediatrics*. Feb; 1980 96(2):209–213. [PubMed: 7351581]
58. Papadimitriou CA, Travlou A, Kalos A, Douratsos D, Lali P. Study of platelet function in patients with sickle cell anemia during steady state and vaso-occlusive crisis. *Acta haematologica*. 1993; 89(4):180–183. [PubMed: 8212998]
59. Wun T, Paglieroni T, Rangaswami A, et al. Platelet activation in patients with sickle cell disease. *British journal of haematology*. Mar; 1998 100(4):741–749. [PubMed: 9531343]
60. Westwick J, Watson-Williams EJ, Krishnamurthi S, et al. Platelet activation during steady state sickle cell disease. *Journal of medicine*. 1983; 14(1):17–36. [PubMed: 6224876]
61. Browne PV, Mosher DF, Steinberg MH, Hebbel RP. Disturbance of plasma and platelet thrombospondin levels in sickle cell disease. *American journal of hematology*. Apr; 1996 51(4): 296–301. [PubMed: 8602630]
62. Foulon I, Bachir D, Galacteros F, Maclof J. Increased in vivo production of thromboxane in patients with sickle cell disease is accompanied by an impairment of platelet functions to the thromboxane A2 agonist U46619. *Arteriosclerosis and thrombosis : a journal of vascular biology / American Heart Association*. Mar; 1993 13(3):421–426. [PubMed: 8443146]
63. Lee SP, Ataga KI, Orringer EP, Phillips DR, Parise LV. Biologically active CD40 ligand is elevated in sickle cell anemia: potential role for platelet-mediated inflammation. *Arteriosclerosis, thrombosis, and vascular biology*. Jul; 2006 26(7):1626–1631.
64. Famodu AA, Oduwa D. Platelet count and platelet factor 3 (PF-3) availability in sickle cell disease. *British journal of biomedical science*. Dec; 1995 52(4):323–324. [PubMed: 8555788]
65. Proenca-Ferreira R, Franco-Penteado CF, Traina F, Saad ST, Costa FF, Conran N. Increased adhesive properties of platelets in sickle cell disease: roles for alphaIIb beta3-mediated ligand binding, diminished cAMP signalling and increased phosphodiesterase 3A activity. *British journal of haematology*. Apr; 2010 149(2):280–288. [PubMed: 20136824]

66. Francis RB Jr. Platelets, coagulation, and fibrinolysis in sickle cell disease: their possible role in vascular occlusion. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. Apr; 1991 2(2):341–353. [PubMed: 1893065]
67. Kenny MW, George AJ, Stuart J. Platelet hyperactivity in sickle-cell disease: a consequence of hyposplenism. *Journal of clinical pathology*. Jul; 1980 33(7):622–625. [PubMed: 7430367]
68. Mehta P. Significance of plasma beta-thromboglobulin values in patients with sickle cell disease. *The Journal of pediatrics*. Dec; 1980 97(6):941–944. [PubMed: 6160233]
69. Semple MJ, Al-Hasani SF, Kioy P, Savidge GF. A double-blind trial of ticlopidine in sickle cell disease. *Thrombosis and haemostasis*. Jul 29; 1984 51(3):303–306. [PubMed: 6388012]
70. Alkjaersig N, Fletcher A, Joist H, Chaplin H Jr. Hemostatic alterations accompanying sickle cell pain crises. *The Journal of laboratory and clinical medicine*. Sep; 1976 88(3):440–449. [PubMed: 956696]
71. Solovey A, Kollander R, Shet A, et al. Endothelial cell expression of tissue factor in sickle mice is augmented by hypoxia/reoxygenation and inhibited by lovastatin. *Blood*. Aug 1; 2004 104(3):840–846. [PubMed: 15073034]
72. Ataga KI. Hypercoagulability and thrombotic complications in hemolytic anemias. *Haematologica*. Nov; 2009 94(11):1481–1484. [PubMed: 19880774]
73. Setty BN, Kulkarni S, Stuart MJ. Role of erythrocyte phosphatidylserine in sickle red cell-endothelial adhesion. *Blood*. Mar 1; 2002 99(5):1564–1571. [PubMed: 11861269]
74. Zwaal RF, Schroit AJ. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*. Feb 15; 1997 89(4):1121–1132. [PubMed: 9028933]
75. Setty BN, Kulkarni S, Dampier CD, Stuart MJ. Fetal hemoglobin in sickle cell anemia: relationship to erythrocyte adhesion markers and adhesion. *Blood*. May 1; 2001 97(9):2568–2573. [PubMed: 11313243]
76. Setty BN, Rao AK, Stuart MJ. Thrombophilia in sickle cell disease: the red cell connection. *Blood*. Dec 1; 2001 98(12):3228–3233. [PubMed: 11719358]
77. Styles L, de Jong K, Vichinsky E, et al. Increased RBC phosphatidylserine exposure in sickle cell disease patients at risk for stroke by transcranial doppler screening. *Blood*. 1997(90):604.
78. Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. *Journal of immunology*. Apr 1; 1992 148(7):2207–2216.
79. Martin SJ, Reutelingsperger CP, McGahon AJ, et al. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. *The Journal of experimental medicine*. Nov 1; 1995 182(5):1545–1556. [PubMed: 7595224]
80. Stuart MJ, Setty BN. Hemostatic alterations in sickle cell disease: relationships to disease pathophysiology. *Pediatric pathology & molecular medicine*. Jan-Feb; 2001 20(1):27–46. [PubMed: 12673843]
81. Kuypers FA, Larkin SK, Emeis JJ, Allison AC. Interaction of an annexin V homodimer (Diannexin) with phosphatidylserine on cell surfaces and consequent antithrombotic activity. *Thrombosis and haemostasis*. Mar 2007; 97(3):478–486. [PubMed: 17334517]
82. Lane PA, O'Connell JL, Marlar RA. Erythrocyte membrane vesicles and irreversibly sickled cells bind protein S. *American journal of hematology*. Dec; 1994 47(4):295–300. [PubMed: 7977302]
83. Schechter AN, Gladwin MT. Hemoglobin and the paracrine and endocrine functions of nitric oxide. *The New England journal of medicine*. Apr 10; 2003 348(15):1483–1485. [PubMed: 12686706]
84. Huang KT, Han TH, Hyduke DR, et al. Modulation of nitric oxide bioavailability by erythrocytes. *Proceedings of the National Academy of Sciences of the United States of America*. Sep 25; 2001 98(20):11771–11776. [PubMed: 11573011]
85. Vaughn MW, Huang KT, Kuo L, Liao JC. Erythrocytes possess an intrinsic barrier to nitric oxide consumption. *The Journal of biological chemistry*. Jan 28; 2000 275(4):2342–2348. [PubMed: 10644684]
86. Butler AR, Megson IL, Wright PG. Diffusion of nitric oxide and scavenging by blood in the vasculature. *Biochimica et biophysica acta*. Sep 16; 1998 1425(1):168–176. [PubMed: 9813307]

87. Morris CR, Kato GJ, Poljakovic M, et al. Dysregulated arginine metabolism, hemolysis-associated pulmonary hypertension, and mortality in sickle cell disease. *JAMA : the journal of the American Medical Association*. Jul 6; 2005 294(1):81–90. [PubMed: 15998894]
88. Villagra J, Shiva S, Hunter LA, Machado RF, Gladwin MT, Kato GJ. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood*. Sep 15; 2007 110(6):2166–2172. [PubMed: 17536019]
89. Kermarrec N, Zunic P, Beloucif S, Benessiano J, Drouet L, Payen D. Impact of inhaled nitric oxide on platelet aggregation and fibrinolysis in rats with endotoxic lung injury. Role of cyclic guanosine 5'-monophosphate. *American journal of respiratory and critical care medicine*. Sep; 1998 158(3):833–839. [PubMed: 9731013]
90. Radomski MW, Palmer RM, Moncada S. The role of nitric oxide and cGMP in platelet adhesion to vascular endothelium. *Biochemical and biophysical research communications*. Nov 13; 1987 148(3):1482–1489. [PubMed: 2825688]
91. Radomski MW, Palmer RM, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet*. Nov 7; 1987 2(8567):1057–1058. [PubMed: 2889967]
92. Gerlach M, Keh D, Bezold G, et al. Nitric oxide inhibits tissue factor synthesis, expression and activity in human monocytes by prior formation of peroxynitrite. *Intensive care medicine*. Nov; 1998 24(11):1199–1208. [PubMed: 9876984]
93. Fiorucci S, Santucci L, Cirino G, et al. IL-1 beta converting enzyme is a target for nitric oxide-releasing aspirin: new insights in the antiinflammatory mechanism of nitric oxide-releasing nonsteroidal antiinflammatory drugs. *Journal of immunology*. Nov 1; 2000 165(9):5245–5254.
94. Dusse LM, Cooper AJ, Lwaleed BA. Tissue factor and nitric oxide: a controversial relationship! *Journal of thrombosis and thrombolysis*. Apr; 2007 23(2):129–133. [PubMed: 17221333]
95. Studt JD, Kremer Hovinga JA, Antoine G, et al. Fatal congenital thrombotic thrombocytopenic purpura with apparent ADAMTS13 inhibitor: in vitro inhibition of ADAMTS13 activity by hemoglobin. *Blood*. Jan 15; 2005 105(2):542–544. [PubMed: 15367436]
96. Schnog JJ, Kremer Hovinga JA, Krieg S, et al. ADAMTS13 activity in sickle cell disease. *American journal of hematology*. Jul; 2006 81(7):492–498. [PubMed: 16755558]
97. Krishnan S, Siegel J, Pullen G Jr, Hevelow M, Dampier C, Stuart M. Increased von Willebrand factor antigen and high molecular weight multimers in sickle cell disease associated with nocturnal hypoxemia. *Thrombosis research*. 2008; 122(4):455–458. [PubMed: 18230405]
98. Zhou Z, Han H, Cruz MA, Lopez JA, Dong JF, Guchhait P. Haemoglobin blocks von Willebrand factor proteolysis by ADAMTS-13: a mechanism associated with sickle cell disease. *Thrombosis and haemostasis*. Jun; 2009 101(6):1070–1077. [PubMed: 19492149]
99. Kuypers FA, de Jong K. The role of phosphatidylserine in recognition and removal of erythrocytes. *Cellular and molecular biology*. Mar; 2004 50(2):147–158. [PubMed: 15095785]
100. Connor J, Pak CC, Schroit AJ. Exposure of phosphatidylserine in the outer leaflet of human red blood cells. Relationship to cell density, cell age, and clearance by mononuclear cells. *The Journal of biological chemistry*. Jan 28; 1994 269(4):2399–2404. [PubMed: 8300565]
101. Schwartz RS, Tanaka Y, Fidler IJ, Chiu DT, Lubin B, Schroit AJ. Increased adherence of sickled and phosphatidylserine-enriched human erythrocytes to cultured human peripheral blood monocytes. *The Journal of clinical investigation*. Jun; 1985 75(6):1965–1972. [PubMed: 4008648]
102. Kato GJ, McGowan V, Machado RF, et al. Lactate dehydrogenase as a biomarker of hemolysis-associated nitric oxide resistance, priapism, leg ulceration, pulmonary hypertension, and death in patients with sickle cell disease. *Blood*. Mar 15; 2006 107(6):2279–2285. [PubMed: 16291595]
103. Nolan VG, Adewoye A, Baldwin C, et al. Sickle cell leg ulcers: associations with haemolysis and SNPs in Klotho, TEK and genes of the TGF-beta/BMP pathway. *British journal of haematology*. Jun; 2006 133(5):570–578. [PubMed: 16681647]
104. Nolan VG, Wyszynski DF, Farrer LA, Steinberg MH. Hemolysis-associated priapism in sickle cell disease. *Blood*. Nov 1; 2005 106(9):3264–3267. [PubMed: 15985542]
105. Jackson N, Franklin IM, Hughes MA. Recurrent priapism following splenectomy for thalassaemia intermedia. *The British journal of surgery*. Aug.1986 73(8):678. [PubMed: 3742187]

106. Macchia P, Massei F, Nardi M, Favre C, Brunori E, Barba V. Thalassemia intermedia and recurrent priapism following splenectomy. *Haematologica*. Sep-Oct;1990 75(5):486–487. [PubMed: 2097271]
107. Boyle S, White RH, Brunson A, Wun T. Splenectomy and the incidence of venous thromboembolism and sepsis in patients with immune thrombocytopenia. *Blood*. Jun 6; 2013 121(23):4782–4790. [PubMed: 23637127]
108. Lovren F, Verma S. Evolving role of microparticles in the pathophysiology of endothelial dysfunction. *Clinical chemistry*. Aug; 2013 59(8):1166–1174. [PubMed: 23529703]
109. Tushuizen ME, Diamant M, Sturk A, Nieuwland R. Cell-derived microparticles in the pathogenesis of cardiovascular disease: friend or foe? *Arteriosclerosis, thrombosis, and vascular biology*. Jan; 2011 31(1):4–9.
110. Shet AS, Aras O, Gupta K, et al. Sick blood contains tissue factor-positive microparticles derived from endothelial cells and monocytes. *Blood*. Oct 1; 2003 102(7):2678–2683. [PubMed: 12805058]
111. Nieuwland R, Berckmans RJ, Rottevel-Eijkman RC, et al. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation*. Nov 18; 1997 96(10):3534–3541. [PubMed: 9396452]
112. Nieuwland R, Berckmans RJ, McGregor S, et al. Cellular origin and procoagulant properties of microparticles in meningococcal sepsis. *Blood*. Feb 1; 2000 95(3):930–935. [PubMed: 10648405]
113. Joop K, Berckmans RJ, Nieuwland R, et al. Microparticles from patients with multiple organ dysfunction syndrome and sepsis support coagulation through multiple mechanisms. *Thrombosis and haemostasis*. May; 2001 85(5):810–820. [PubMed: 11372673]
114. Berckmans RJ, Nieuwland R, Tak PP, et al. Cell-derived microparticles in synovial fluid from inflamed arthritic joints support coagulation exclusively via a factor VII-dependent mechanism. *Arthritis and rheumatism*. Nov; 2002 46(11):2857–2866. [PubMed: 12428225]
115. Lok CA, Nieuwland R, Sturk A, et al. Microparticle-associated P-selectin reflects platelet activation in preeclampsia. *Platelets*. Feb; 2007 18(1):68–72. [PubMed: 17365856]
116. Gerotziafas GT, Van Dreden P, Chaari M, et al. The acceleration of the propagation phase of thrombin generation in patients with steady-state sickle cell disease is associated with circulating erythrocyte-derived microparticles. *Thrombosis and haemostasis*. Jun; 2012 107(6):1044–1052. [PubMed: 22535498]
117. Patel RK, Arya R. Tests for hereditary thrombophilia are of limited value in the black population. *Stroke; a journal of cerebral circulation*. Dec.2003 34(12):e236.
118. Mack R, Chowdary D, Streck D, Dermody J. Inherited thrombophilia genes in minorities. *Genetic testing*. 1999; 3(4):371–373. [PubMed: 10627946]
119. Helley D, Besmond C, Ducrocq R, et al. Polymorphism in exon 10 of the human coagulation factor V gene in a population at risk for sickle cell disease. *Human genetics*. Aug; 1997 100(2):245–248. [PubMed: 9254858]
120. Kahn MJ, Scher C, Rozans M, Michaels RK, Leissinger C, Krause J. Factor V Leiden is not responsible for stroke in patients with sickling disorders and is uncommon in African Americans with sickle cell disease. *American journal of hematology*. Jan; 1997 54(1):12–15. [PubMed: 8980255]
121. Kahn JE, Veyssier-Belot C, Renier JL, de Mazancourt P, Peltier JY, de Raucourt E. Recurrent thromboembolism in a patient with beta-thalassemia major associated with double heterozygosity for factor V R506Q and prothrombin G20210A mutations. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. Jul; 2002 13(5):461–463. [PubMed: 12138375]
122. Wright JG, Cooper P, Malia RG, et al. Activated protein C resistance in homozygous sickle cell disease. *British journal of haematology*. Mar; 1997 96(4):854–856. [PubMed: 9074431]
123. Moreira Neto F, Lourenco DM, Noguti MA, et al. The clinical impact of MTHFR polymorphism on the vascular complications of sickle cell disease. *Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica ... [et al]*. Oct; 2006 39(10):1291–1295.

124. Andrade FL, Annichino-Bizzacchi JM, Saad ST, Costa FF, Arruda VR. Prothrombin mutant, factor V Leiden, and thermolabile variant of methylenetetrahydrofolate reductase among patients with sickle cell disease in Brazil. *American journal of hematology*. Sep; 1998 59(1):46–50. [PubMed: 9723576]
125. Kordes U, Janka-Schaub G, Schneppenheim R. Homozygous Factor V Leiden mutation in sickle cell anaemia. *British journal of haematology*. Jan.2002 116(1):236. [PubMed: 11848090]
126. Fawaz NA, Bashawery L, Al-Sheikh I, Qatari A, Al-Othman SS, Almawi WY. Factor V-Leiden, prothrombin G20210A, and MTHFR C677T mutations among patients with sickle cell disease in Eastern Saudi Arabia. *American journal of hematology*. Jul; 2004 76(3):307–309. [PubMed: 15224376]
127. Zimmerman SA, Ware RE. Inherited DNA mutations contributing to thrombotic complications in patients with sickle cell disease. *American journal of hematology*. Dec; 1998 59(4):267–272. [PubMed: 9840906]
128. Zeinali S, Duca F, Zarbakhsh B, Tagliabue L, Mannucci PM. Thrombophilic mutations in Iran. *Thrombosis and haemostasis*. Feb; 2000 83(2):351–352. [PubMed: 10739401]
129. Rahimi Z, Vaisi-Raygani A, Nagel RL, Muniz A. Thrombophilic mutations among Southern Iranian patients with sickle cell disease: high prevalence of factor V Leiden. *Journal of thrombosis and thrombolysis*. Jun; 2008 25(3):288–292. [PubMed: 17619828]
130. Isma'eel H, Arnaout MS, Shamseddeen W, et al. Screening for inherited thrombophilia might be warranted among Eastern Mediterranean sickle-beta-0 thalassemia patients. *Journal of thrombosis and thrombolysis*. Oct; 2006 22(2):121–123. [PubMed: 17008978]
131. Otrrock ZK, Mahfouz RA, Taher AT. Should we screen Eastern Mediterranean sickle beta-thalassemia patients for inherited thrombophilia? *Journal of thrombosis and haemostasis : JTH*. Mar; 2005 3(3):599–600. [PubMed: 15748263]
132. Koren A, Zalman L, Levin C, Abu Hana M, Mader R, Shalev S. Venous thromboembolism, factor V Leiden, and methylenetetrahydrofolate reductase in a sickle cell anemia patient. *Pediatric hematology and oncology*. Sep-Oct;1999 16(5):469–472. [PubMed: 10505325]
133. Bray PF. Platelet glycoprotein polymorphisms as risk factors for thrombosis. *Current opinion in hematology*. Sep; 2000 7(5):284–289. [PubMed: 10961578]
134. Furihata K, Nugent DJ, Kunicki TJ. Influence of platelet collagen receptor polymorphisms on risk for arterial thrombosis. *Archives of pathology & laboratory medicine*. Mar; 2002 126(3):305–309. [PubMed: 11860304]
135. Deckmyn H, Ulrichts H, Van De Walle G, Vanhoorelbeke K. Platelet antigens and their function. *Vox sanguinis*. Jul; 2004 87(Suppl 2):105–111. [PubMed: 15209892]
136. Castro V, Alberto FL, Costa RN, et al. Polymorphism of the human platelet antigen-5 system is a risk factor for occlusive vascular complications in patients with sickle cell anemia. *Vox sanguinis*. Aug; 2004 87(2):118–123. [PubMed: 15355504]
137. Al-Subaie AM, Fawaz NA, Mahdi N, et al. Human platelet alloantigens (HPA) 1, HPA2, HPA3, HPA4, and HPA5 polymorphisms in sickle cell anemia patients with vaso-occlusive crisis. *European journal of haematology*. Dec 1; 2009 83(6):579–585. [PubMed: 19702628]
138. Wun T, Soulieres D, Frelinger AL, et al. A double-blind, randomized, multicenter phase 2 study of prasugrel versus placebo in adult patients with sickle cell disease. *Journal of hematology & oncology*. 2013; 6:17. [PubMed: 23414938]
139. Clotz I, Tam J. Acetylation of sickle cell hemoglobin by aspirin. *Proceedings of the National Academy of Sciences of the United States of America*. 1973(70):1313–1315.
140. Osamo NO, Photiades DP, Famodu AA. Therapeutic effect of aspirin in sickle cell anaemia. *Acta haematologica*. 1981; 66(2):102–107. [PubMed: 6794308]
141. Greenberg J, Ohene-Frempong K, Halus J, Way C, Schwartz E. Trial of low doses of aspirin as prophylaxis in sickle cell disease. *The Journal of pediatrics*. May; 1983 102(5):781–784. [PubMed: 6842340]
142. Zago MA, Costa FF, Ismael SJ, Tone LG, Bottura C. Treatment of sickle cell diseases with aspirin. *Acta haematologica*. 1984; 72(1):61–64. [PubMed: 6433636]

143. Chaplin H Jr, Alkjaersig N, Fletcher AP, Michael JM, Joist JH. Aspirin-dipyridamole prophylaxis of sickle cell disease pain crises. *Thrombosis and haemostasis*. Jul 15; 1980 43(3):218–221. [PubMed: 7006142]
144. Cabannes R, Lonsdorfer J, Castaigne JP, Ondo A, Plassard A, Zohoun I. Clinical and biological double-blind-study of ticlopidine in preventive treatment of sickle-cell disease crises. *Agents and actions Supplements*. 1984; 15:199–212. [PubMed: 6385647]
145. Salvaggio JE, Arnold CA, Banov CH. Long-term anti-coagulation in sickle-cell disease. A clinical study. *The New England journal of medicine*. Jul 25.1963 269:182–186. [PubMed: 13991207]
146. Schnog JB, Kater AP, Mac Gillavry MR, et al. Low adjusted-dose acenocoumarol therapy in sickle cell disease: a pilot study. *American journal of hematology*. Nov; 2001 68(3):179–183. [PubMed: 11754399]
147. Ahmed S, Siddiqui AK, Iqbal U, et al. Effect of low-dose warfarin on D-dimer levels during sickle cell vaso-occlusive crisis: a brief report. *European journal of haematology*. Mar; 2004 72(3):213–216. [PubMed: 14962240]
148. Qari MH, Aljaouni SK, Alardawi MS, et al. Reduction of painful vaso-occlusive crisis of sickle cell anaemia by tinzaparin in a double-blind randomized trial. *Thrombosis and haemostasis*. Aug; 2007 98(2):392–396. [PubMed: 17721622]
149. De Franceschi L, Cappellini MD, Olivieri O. Thrombosis and sickle cell disease. *Seminars in thrombosis and hemostasis*. Apr; 2011 37(3):226–236. [PubMed: 21455857]
150. Ataga KI, Key NS. Hypercoagulability in sickle cell disease: new approaches to an old problem. *Hematology / the Education Program of the American Society of Hematology American Society of Hematology Education Program*. 2007:91–96. [PubMed: 18024615]
151. Wolters HJ, ten Cate H, Thomas LL, et al. Low-intensity oral anticoagulation in sickle-cell disease reverses the prethrombotic state: promises for treatment? *British journal of haematology*. Jul; 1995 90(3):715–717. [PubMed: 7647016]

- 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 al¹⁴⁹

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 Dipyridamole	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/S β 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/S β thalassemia	Randomized (29)	Aspirin vs. Placebo	No change in pain episodes or laboratory values
Wun et al ¹³⁸	HbSS/S β 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 Key¹⁵⁰

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 al 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 Key¹⁵⁰