The genetic and molecular basis of sickle cell disease (SCD) has long been characterized but the pathophysiological basis has not been entirely defined. How a red cell hemolytic disorder initiates inflammation, endothelial dysfunction, coagulation activation, and eventually leads to vascular thrombosis, is yet to be elucidated. Recent evidence has demonstrated a high frequency of unprovoked/recurrent venous thromboembolism (VTE) in SCD, with an increased risk of mortality among patients with a history of VTE. Here, we provide an in-depth review of the molecular basis for the prothrombotic state in SCD, specifically highlighting emerging evidence for activation of overlapping inflammation and coagulation pathways that predispose to venous thromboembolism. We share perspectives in managing venous thrombosis in SCD, highlighting innovative therapies with the potential to influence the clinical course of disease and reduce thrombotic risk, while maintaining an acceptable safety profile.

The molecular basis for the prothrombotic state in sickle cell disease

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Introduction

Renewed interest in sickle cell disease (SCD) as a significant global health problem by academia, industry and policy makers is leading the resurgence of efforts to treat or cure patients with this disease. Drug development has recently led to US Food and Drug Administration (FDA) approval of three new agents (L-glutamine, crizanlizumab, voxelotor) adding much needed diversity to the hitherto lone disease-modifying therapy, hydroxyurea (HU). Moreover, advanced phase clinical trials of molecules targeting diverse disease mechanisms, if efficacious, could ameliorate the protean manifestations of SCD. In addition, curing SCD either through stem cell transplantation or gene therapy has become a reality for a small number of patients. Given the October 2019 joint National Institutes of Health (NIH)-Bill and Melinda Gates Foundation funding declaration, inclusion of patients from high disease burden resource-scarce settings in such trials of novel curative therapies seems plausible. Prospects appear particularly promising for SCD patients in resource-scarce settings worldwide, where the need is greatest. Despite these advances, many patients with SCD continue to experience severe complications, and understanding the pathophysiology of “downstream” events remains important for the development of therapies to target specific complications. One of these phenomena is the sickle prothrombotic state that is largely believed to be responsible for both arterial and venous thrombosis in SCD. In its most devastating form, thrombosis occurs in arteries leading to overt stroke and silent cerebral infarction in SCD patients as early as childhood. In adulthood, SCD patients develop deep venous thrombosis (DVT) and pulmonary embolism (PE), collectively termed venous thromboembolism (VTE). Thrombotic vasculopathy in SCD is accompanied by significant organ dysfunction, morbidity from diminished quality of life, and mortality. Yet, how the complex pathobiology initiated by sickle RBC-mediated endothelial inflammation/dysfunction and coagulation activation leads to vessel injury, leakage, and vascular thrombosis remains to be clarified. At the mechanistic level, there is a scientific gap in our understanding of coagulation-mediated pathologies of SCD, a fact noted in the National Heart, Lung, and Blood Institute (NHLBI) evidence-based guidelines, which makes this an active area of research. Therefore, gaining insight into the patho-
physiological basis for the prothrombotic state in patients with SCD and how one might attenuate thrombotic risk is of the utmost importance. Studies of vascular pathobiology using in vitro and animal models of SCD have identified both insightful and discrepant findings, when compared with human SCD, as noted in the accompanying review in this issue of the Journal by Conan and De Paula.10 Here we examine the molecular basis for the prothrombotic state and establish the scientific rationale to target dysregulated coagulation pathways in patients with SCD. For a more comprehensive overview of arterial thrombosis and/or SCD management, the reader is referred to recent publications in the literature.11-13

**Sickle cell disease: an acquired hypercoagulable state**

The underlying mechanism(s) for the prothrombotic tendency in SCD has been the subject of speculation for many decades.14,15 Several clinical observations suggest that the presence of sickle hemoglobin (HbS) is either directly or indirectly associated with the development of thrombotic complications in SCD. The early age of onset of arterial complications such as stroke and silent cerebral infarction in children with hemoglobin SS disease, in particular, is a clear example of the thrombotic potential associated with hemoglobin S. Furthermore, in adults, a wide range of sickling hemoglobinopathies have been associated with the development of VTE, from hemoglobin SS to compound heterozygous states to sickle cell trait (SCT).

Numerous epidemiological studies of VTE in SCD have verified an increased risk of this complication (Table 1). A retrospective analysis of the Cooperative Study of SCD demonstrated a 11.3% cumulative incidence of VTE by the age of 40,16 and a similarly high incidence was found in a large California discharge database.17 These estimates are particularly striking when compared to population studies of patients with thrombophilia, as the incidence rates of VTE observed in SCD are similar to those found in families with high-risk thrombophilia such as protein C and S deficiency.18 In addition, after controlling for confounding factors such as hospitalization, studies have found that VTE rates are high even among SCD with a lower number of hospitalizations,8 and that VTE prevalence in pregnant women with SCD increases approximately 5-fold compared to non-SCD pregnant women.17,18 Furthermore, the recurrence rate of VTE in SCD has also been noted to be exceedingly high (>35% at 5 years),8,16 another indication of the persistent thrombogenic potential in SCD.

Further confirmation of the relationship between the presence of HbS and a prothrombotic state are observations noted in healthy individuals with SCT. In two large population-based studies, VTE risk in individuals with SCT was almost 2-fold higher than ethnic-matched counterparts, despite the absence of either clinical sickling symptoms or HbS percentages encountered in patients with SCD (Table 1).19,20 As with the known pathophysiology of SCD complications, the presence of HbS alone is unlikely to be the sole contributor to thrombotic risk. Additional mechanisms, described further below, are likely to contribute to the acquired prothrombotic state in SCD.

**Clinical and genetic risk factors for the prothrombotic state**

Clinical and biological factors provide insight into why a genetic disorder primarily involving red cells sets off a cascade of events leading to an acquired prothrombotic state. Risk factors for VTE in SCD include increased disease severity (as measured by averaging ≥3 hospital admissions a year for VOC), exposure to erythropoiesis stimulating agents or blood transfusion, insertion of central venous catheters (CVC), surgical splenectomy, and hospitalization.21,22 As mentioned above, not only are SCD patients at a high risk for developing early onset VTE, but their risk for VTE recurrence after an index VTE has been noted to be high. Clinical risk factors for VTE recurrence include averaging >3 hospital admissions a year, lower extremity DVT as the index event, and a prior history of pneumonia/acute chest syndrome (ACS).23,24 Incomplete adherence to anticoagulation is also likely to contribute to higher recurrence rates, though this has not been formally evaluated. In a single center retrospective study, use of direct oral anticoagulants (DOAC) was associated with lower recurrence rate.22

Genotype may also modify thrombotic risk in SCD. Certainly, children with HbSS/Sα are at highest risk for arterial thrombosis such as stroke.25 In terms of VTE, the influence of genotype has not been fully elucidated, with some studies demonstrating an increased risk of VTE among sickle variant syndromes and others showing the highest risk among HbSS/Sβ.6,16 The influence of co-inheritance of either α-thalassemia or gene modifiers regulating human fetal hemoglobin expression15-27 or both, has not been studied for VTE but may attenuate stroke risk.3

The influence of heritable thrombophilia mutations on VTE risk in SCD remains to be completely determined. Genetic variants, factor V Leiden and prothrombin FII G20210A in particular explain up to 50% of unprovoked VTE among Caucasians.26 Notably, VTE risk is 5-fold higher in individuals reporting African descent compared with those reporting Asian descent, whereas white individuals have only an intermediate-level risk.27 However, factor V Leiden and prothrombin FII G20210A have low allele frequencies among individuals reporting African descent and are not associated with venous thrombosis in SCD.28-31 Thus, other heritable or acquired thrombophilia-associated mutations could explain thrombotic risk in SCD. One recent study identified two known thrombomodulin gene variants (THBD rs2567617 and rs1998081) that were associated with arterial and venous thrombosis in SCD patients.21 A recent genome-wide association study conducted among African Americans in the general population identified three novel intronic gene variations (LEMD3, LY86, LOC100130298) associated with higher odds of developing VTE.28 LEMD3 encodes for a nuclear membrane protein that interacts with transforming growth factor (TGF)-β to downregulate the activation of TGF-β target genes30-32 and LY86 encodes for MD-1, which regulates expression of a cell surface protein homologous to toll-like receptor (TLR) 4.32-34 Given the involvement of the innate immune system in both VTE35 and the vascular pathobiology of SCD,29 genetic dysregulation of these pathways in SCD patients could influence thrombophilia. Acquired mutations also influence thrombotic risk; JAK2,30,31 the most frequent age-induced clonal hematopoietic mutation is associated with increased
venous thrombotic events. Future studies may clarify the role of these heritable or acquired mutations.

**Thrombo-inflammatory processes in sickle cell disease that alter hemostatic balance**

A triad of thrombosis risk factors first described by Virchow that includes increased blood coagulability, altered blood flow (stasis) and endothelial dysfunction, are all evident in SCD. The endothelium, a critical regulator of thrombo-inflammatory processes maintains vascular health by exerting anti-coagulant, anti-inflammatory, and anti-platelet actions. The sickle proinflammatory state leads to endothelial dysfunction, thereby shifting the hemostatic balance towards a prothrombotic state (Figure 1). Accumulated evidence suggests that SCD is a thrombo-inflammatory disorder as reflected by alterations in components of coagulation and inflammatory pathways. This is most clearly demonstrated in the acute exaggerated thrombo-inflammatory response that accompanies ischemia-reperfusion (IR) injury in SCD patients. Endothelial inflammation leads to surface expression of adhesion molecules (P-selectin and E-selectin) and release of prothrombotic granule contents (von Willebrand factor and FVIII), both effects enhancing leukocyte/platelet adhesion (Figure 2). Repeated VOC episodes amongst other pathophysiology leads to hemolysis and subsequent release of cell-free heme/hemoglobin. By activating converging inflammatory pathways, such as TLR signaling, NETosis/neutrophil extracellular trap (NET) formation and priming the inflammasome, cell-free heme amplifies inflammation (Figures 1 and 2). Inflammation, shear stress and hypoxia, which are common phenomena in VOC, under experimental conditions can induce abnormal endothelial TF gene and protein expression. SCD patients have a high prevalence of PE (0.44%) compared with African-Americans without SCD (0.12%). DVT prevalence was similar in both groups, 0.44% and 0.40%, respectively.

### Table 1. Epidemiological studies of venous thromboembolism (VTE) in sickle cell disease (SCD) and sickle cell trait (SCT).

<table>
<thead>
<tr>
<th>Author and ref.</th>
<th>Study aim, design and setting</th>
<th>Main findings</th>
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<tbody>
<tr>
<td>Stein et al.(^{12})</td>
<td>Aim: Determine frequency and prevalence of PE and DVT. Retrospective analysis of National Hospital Discharge Survey comparing SCD patients ≥40 years vs. African-American patients.</td>
<td>SCD patients have a high prevalence of PE (0.44%) compared with African-Americans without SCD (0.12%). DVT prevalence was similar in both groups, 0.44% and 0.40%, respectively.</td>
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<tr>
<td>Austin et al.(^{12})</td>
<td>Aim: Evaluate the incidence of VTE in individuals with SCT. Case-control study of N=515 hospitalized patients vs. 555 controls.</td>
<td>The risk of VTE is increased approx. 2-fold among SCT patients compared with controls.</td>
</tr>
<tr>
<td>Naik et al.(^{16})</td>
<td>Aim: Define frequency and characteristics of VTE in SCD. Retrospective single center study of N=404 SCD patients (Sickle Cell Center for Adults-Johns Hopkins).</td>
<td>VTE is common (25%), occurring at a mean age of 30 years. Sickle variants genotypes and tricuspid regurgitant jet velocity ≥2.5 m/s were associated with non-catheter-related VTE.</td>
</tr>
<tr>
<td>Seaman et al.(^{18})</td>
<td>Aim: Evaluate rates and risk factors related with VTE in pregnant women with SCD. Retrospective using Pennsylvania Health Care Database.</td>
<td>Pregnancy-related VTE in women with SCD appeared to be 1.5-5 times greater than pregnancy-related VTE in the general population. N=212 SCD pregnant women.</td>
</tr>
<tr>
<td>Naik et al.(^{4})</td>
<td>Aim: Determine the incidence of first VTE. Retrospective cohort study using prospective data from the co-operative study of SCD. N=1,523 SCD patients ≥15 years.</td>
<td>The cumulative incidence for first VTE was 11.3% by age 40 years. Incidence of PE exceeded that of isolated DVT. SCD patients with VTE had a higher mortality than those without VTE.</td>
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<tr>
<td>Folsom et al.(^{8})</td>
<td>Aim: Risk of VTE in individuals with SCT. Prospective population-based cohort (1987-2011). N=268 SCT middle-aged patients.</td>
<td>SCT carries a 2-fold increased risk of PE but did not appear to be associated with elevated VTE risk.</td>
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<tr>
<td>Little et al.(^{18})</td>
<td>Aim: Determine risk of VTE in individuals with SCT. Prospective cohort study with nested case-control design. N=6,758</td>
<td>SCT individuals had a higher risk of VTE, particularly PE, compared with non-carriers.</td>
</tr>
<tr>
<td>Brunson et al.(^{9})</td>
<td>Aim: Evaluate VTE incidence in SCD. Retrospective study using California administrative database. N=6,237 SCD patients.</td>
<td>The cumulative incidence of VTE was high (11.2%) in SCT patients. The occurrence of VTE was associated with higher mortality.</td>
</tr>
<tr>
<td>Kumar et al.(^{10})</td>
<td>Aim: Evaluate the VTE incidence in children with SCD. Pediatric health information database. N=181.</td>
<td>1.7% developed VTE, use of a central venous catheter was associated with VTE development, and VTE was associated with mortality.</td>
</tr>
<tr>
<td>Brunson et al.(^{11})</td>
<td>Aim: Determine VTE recurrence and bleeding risk in SCD patients with index VTE. Retrospective study using California administrative database. N=877 SCD patients with an index VTE.</td>
<td>The cumulative incidence of VTE recurrence was 13.2% and 24.1% at 1-year and 5-year follow-up. The cumulative incidence of bleeding was 4.9% and 7.9% at 6 months and 1 year following an incident VTE.</td>
</tr>
<tr>
<td>Srisuwananukorn et al.(^{12})</td>
<td>Aim: Investigate the genetic and clinical predictors of arterial and venous thrombosis in SCD. Longitudinal single center cohort study. N=1,193 pediatric and adult SCD patients.</td>
<td>VTE risk was independently associated with HbSS/Sb genotype, HU use, lower estimated glomerular filtration rate, and higher Hb and WBC count. THBD variants, rs2567617 and rs1988081 were associated with thrombosis.</td>
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PE: pulmonary embolism; DVT: deep vein thromboses; N: number; HU: hydroxyurea; THBD: thrombomodulin; Hb: hemoglobin; WBC: white blood cell.
patients demonstrate abnormally elevated levels of intravascular TF that is believed to trigger activation of coagulation and pathological thrombosis.\textsuperscript{52-55} Critically, intravascular TF binding with factor VIIa activates the extrinsic pathway of coagulation by converting coagulation factor X to Xa and generating thrombin (Figure 3), which, unchecked, leads to vascular fibrin deposition. From this perspective, defining the molecular mechanisms regulating these thrombo-inflammatory processes in SCD and identifying interventions to counter vascular thrombosis is of major relevance.

\textbf{Cellular components of blood that facilitate thromboinflammation}

\textbf{Sickle red cells}

That red cells in SCD are likely to be involved in thrombus formation is supported by the relationship between hematocrit and VTE,\textsuperscript{24} possibly resulting from alterations in viscosity, adhesive cellular interactions, and microvascular stasis.\textsuperscript{56,57} Studies of sickle red cells have identified numerous receptors and ligands that mediate adhesive interactions with the vessel wall, implicating their role in

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\textbf{Prothrombotic state in sickle cell disease}

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\begin{figure}[h]
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\caption{Sickle hemoglobin (HbS) polymerization, hemolysis and ischemia/reperfusion injury induce chronic inflammation. (A) Primary to sickle cell disease pathology is polymerization of HbS during red cell deoxygenation that results in sickled red cells and frequent painful vaso-occlusive crises. Hypoxia in the venous vasculature and valve pockets may worsen sickling mediated hemolysis and venous endothelial injury/inflammation. (B) Repeated sickling and unsickling episodes lead to intravascular hemolysis and release of free heme that consumes nitric oxide (NO). Sickle RBC also activate neutrophils among other cells, forming hetero and homotypic aggregates with blood cells that lead to vaso-occlusion in the post capillary venule. Endothelial damage and endothelial surface expression of adhesion and procoagulant molecules leads to transit delays increasing the potential for stasis and further sickling. Damage-associated molecular pattern (DAMP) molecules (free heme and high mobility group box 1 [HMGB1]) activate of various inflammatory pathways, e.g. NETosis, toll-like receptor (TLR) signaling, innate immune response and production of reactive oxidative species (ROS) lead to chronic inflammation. Repeated episodes of vaso-occlusive crisis (VOC) leads to ischemia followed by reperfusion mediating a well characterized injury response in the vascular endothelium. Figure created with BioRender.com. VCAM1: vascular cell adhesion molecule 1; EV TF: extracellular vesicle tissue factor; NET: neutrophil extracellular trap.}
\end{figure}
post capillary venule occlusion (Figure 2). Elevated numbers of phosphatidylserine (PS) positive sickle red cell and red cell derived extracellular vesicles (EV) are observed in SCD patients, which correlate with markers of thrombin generation. In non-SCD models, venous thrombus size is impacted by localization of red cells within blood clots, which in turn is affected by fibrin network density and fibrin α-chain crosslinking activity. Recent studies in SCD mice have shown higher amounts of fibrin deposition and red cell entrapment within the developing venous thrombus that are likely to impact thrombus structure and stability. These findings imply that venous clots in SCD patients and individuals with SCT are more friable and prone to embolization, possibly explaining why individuals with sickling hemoglobinopathies appear to have a higher risk of PE compared to DVT. However, in situ pulmonary thrombosis in SCD patients suggests heterogenous mechanisms, that may include abnormal pulmonary vascular endothelial TF expression.

Platelets

Due to the physiological role platelets play in primary hemostasis, platelet activation probably contributes to thrombosis in SCD. For example, activated platelets in SCD can recruit leukocytes to sites of inflammation through surface P-selectin, and release prothrombotic granule contents. Besides, activated platelets form homotypic and heterotypic cell-aggregates, and platelet-neutrophil aggregates contribute to pulmonary arteriolar micro emboli. Recent studies, both murine models and SCD patients have consistently demonstrated activation of the platelet NLRP3 inflammasome, suggesting an autocrine feedback loop for IL-1β driven priming of innate immune and vascular endothelial cells. Moreover, solu-
ble CD40L and thrombospondin-1, two platelet-derived molecules are reportedly elevated in SCD patients with a history of ACS, which, in the light of dense platelet-rich thrombi found on autopsy in the pulmonary vasculature of over half of ACS patients, provides evidence for platelet-mediated thrombosis.

**Leukocytes**

Innate immune cells mediate SCD pathophysiology, as recently reviewed in this Journal. Amongst these, monocytes and neutrophils facilitate thrombotic vasculopathy by directly inducing endothelial injury and activating coagulation. Neutrophils undergo NETosis, a neutrophil defense mechanism that involves the release of NET and initiates venous thrombosis. In vitro studies suggest that actually cell-free DNA (cfDNA) and histones, rather than intact NET, activate coagulation, and while SCD patients have elevated plasma levels of cfDNA, histones and NET, no direct evidence links them with VTE development. Neutrophil-endothelial and neutrophil-platelet cross-talk, mediated via P-selectin-PSGL1 interactions are key aspects of SCD pathobiology, as evidenced by the clinical efficacy of the anti-P-selectin antibody crizanlizumab (Figure 2). Monocytes regulate important aspects of blood coagulation, innate immune response, reticuloendothelial function, and phagocytosis. Circulating TF and TF monocyte EV form the major fraction of measurable blood borne TF and thus contribute meaningfully to coagulation abnormalities observed in SCD patients. Monocyte activation by heme and placental growth factor (PLGF) released from sickle red cells can stimulate the production of proinflammatory cytokines and chemokines (e.g., IL-1β, TNF-α, MCP-1 and MIP-1β), that in turn are capable of inducing TF gene expression. Lastly, frequent VOC in SCD could reduce the number of patrolling monocytes (CD14<sup>+/-</sup>CD16<sup>-</sup>) responsible for restoration of endothelial function thereby augmenting endothelial injury and dysfunction.

**Molecular components in blood facilitating thromboinflammation**

**Blood borne tissue factor**

As described above, intravascular TF expression in the setting of SCD-related endothelial damage is likely a major contributor to the hypercoagulable state of SCD; however, the role of "blood borne" TF in SCD-related thrombosis is largely unknown. Studies have shown

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Figure 3. The TF and contact pathways activate coagulation and generate thrombin. Abnormal expression of intravascular TF in sickle cell disease (SCD), the so called "blood borne TF," triggers intravascular blood coagulation and pathological thrombosis. After binding with plasma factor VIIa, TF activates the extrinsic pathway of coagulation, generating thrombin, which mediates fibrin deposition, thrombosis and vascular remodeling. Besides lowered natural anticoagulant factor levels (thrombomodulin, protein C and S) also favor thrombosis. Activation of the contact pathway of coagulation by RBC phosphatidylserine (PS) exposure, cell-derived extracellular vesicles (EV), platelet polyphosphates, cfDNA and NET also sustain thrombin generation. TF and thrombin cause chronic inflammation possibly leading to endothelial injury, vascular permeability, angiogenesis and vascular remodeling, all of which are reflected by vasculopathy. Figure created with BioRender.com.
that SCD patients have elevated blood borne TF procoagulant activity. Specifically, TF EV, which are derived from monocytes and endothelial cells, have been found to be elevated in SCD patients during acute VOC. Although, frequent VOC in SCD has been associated with an increased risk of VTE,5 no studies have evaluated whether cell-surface TF expression or TF EV play a direct role in initiating venous thrombosis.

Several other regulatory mechanisms could modify VTE risk in SCD, as not all SCD patients with elevated levels of blood borne TF experience pathological thrombosis. For example, inactivation of blood borne TF by tissue factor pathway inhibitor (TFPI) probably protects against pathological thrombosis,51 but TFPI antigen levels in SCD patients are found to be normal.52 Besides, the procoagulant activity of TF is under post-translational regulatory control; these mechanisms are beyond the scope of this discussion but are reviewed elsewhere.92 Regulation of TF procoagulant activity could limit overt clinical thrombosis in SCD but does not appear sufficient to inhibit TF and thrombin-mediated inflammatory vasculopathy.53-55

**Thrombin generation**

Activation of coagulation either through the TF or contact factor pathways leads to generation of thrombin. Thrombin, one of the most potent biological proinflammatory molecules, activates both cellular and plasmatic components of blood. These actions primarily include cleavage of factors comprising the plasma coagulation, complement and fibrinolytic cascades. In addition to converting soluble fibrinogen to insoluble fibrin, thrombin activates complement, innate immune cells, the endothelium and platelets, which in SCD mediates vascular thrombosis and vessel wall remodeling (Figure 3).41,45 Moreover, overwhelming evidence for thrombin-mediated vasculopathy is derived from elegant studies in sickle mouse models.56,57

In the aftermath of the initial thrombin burst generated by the TF-VIIa complex, a continuous supply of thrombin generation is required for pathological thrombosis. Thrombin generation is sustained in SCD patients even during the steady state, as demonstrated by the observation of elevated plasma levels of thrombin-anti thrombin complexes, D-dimers, and prothrombin fragment 1.295-97 In SCD, thrombin generation is dysregulated in vivo,98,99 which may increase “delay time”114 augmenting the fraction of polymersized HbS; and (ii) formation of hetero and homotypic cellular aggregates that, coupled with pathways, such as TLR signaling, NETosis (see above) and activation of the inflamasome.41 Murine studies that delineate how prototypic DAMP molecules (e.g., cell-free hemoglobin and high-mobility group box 1 [HMGB1]) play a critical role in VTE pathophysiology offer major insight into the occurrence of thrombosis in SCD.43,50,102-105

As noted above, cell-free hemoglobin is proinflammatory and prothrombotic due to nitric oxide (NO) consumption,106 induction of endothelial surface TF70 and TLR4-induced monocyte priming.46 Similarly, disulfide HMGB1, derived from platelets, prime the leukocyte inflamma- some and induce NET, facilitating VTE development in a murine model.107 Plasma HMGB1 levels are elevated in patients with SCD,72,108 but their contribution to NET formation and VTE pathophysiology in SCD is uncertain. Murine models of intravascular hemolysis demonstrate a cooperative role for complement activation and P-selectin in mediating thrombotic injury of hepatic and renal vascular endothelium, and provide insight into how sickle hemolysis might mediate organ injury.45,109 Taken together with the findings that HU attenuates complement activation in SCD patients and the efficacy of P-selectin blockade in preventing VOC,85 it is apparent that modulating these pathways may offset microvascular thrombosis. Advancing our understanding of these complex interactions between dysregulated inflammation and coagulation processes in SCD might offer additional insights and identify new therapeutic targets to limit thrombosis, particularly VTE.

**Thromboinflammation, vascular injury and vasculopathy**

In addition to pathological thrombosis occurring as a result of the TF/VIIa complex (as described above), cell surface TF expression leads to inflammation. TF-mediated inflammation occurs either via the effects of downstream coagulation proteases (see above) or other vascular endothelial and blood cells98 or intracellularly, via its cytoplasmic tail.90 Thrombin’s subsequent interaction with endothelial cell surface protease activating receptors (PAR) accentuates vascular endothelial inflammation in SCD.110 Continued activation of coagulation, initially triggered by TF, likely occurs via EV and PS positive red cells in circulating blood, that by virtue of their surface PS content support the assembly of tenase and prothrombinase complexes (Figures 1 and 3). Moreover, in addition to procoagulant effects exerted by surface TF and PS, EV display membrane surface antigens, e.g., P-selectin, that engage with ligands to enhance production of TF EV112,113 and reactive oxygen species (ROS).110 Splenic hypofunction and diminished reticuloendothelial clearance in SCD lead to accumulation of prothrombotic mediators, i.e., PS; red cells, red cell derived EV and leukocyte/endothelial derived TF- EV. In venous vascular beds prone to stasis and hypoxia, accumulation of procoagulant factors may overwhelm anticoagulant defenses and lead to pathological thrombosis.

A likely sequence of events may include the following: (i) sluggish cell transit through the post capillary venules which may increase “delay time” augmenting the fraction of polymerized HbS; and (ii) formation of hetero and homotypic cellular aggregates that, coupled with...
increased endothelial cell adherence promote stasis, lead to further red cell sickling, vaso-occlusion, and ischemic crisis. These sickling cycles promote repeated vascular injury, vasomotor dysfunction, chronic inflammation and vasculopathy in patients with SCD.\textsuperscript{113,116} Moreover, the cumulative effects of stasis, altered rheology, vascular fibrin deposition and chronic inflammation ultimately leads to chronic vasculopathy\textsuperscript{97} and characterizes the multiorgan failure observed in SCD patients.\textsuperscript{117} Vasculopathy in patients experiencing DVT is reflected by the onset of post-thrombotic syndrome, valvular venous insufficiency or leg ulceration and, in patients experiencing PE, by the onset of chronic thromboembolic pulmonary hypertension (CTEPH)\textsuperscript{118}. The sickle prothrombotic state and resulting VTE, therefore, results in considerable cardiopulmonary morbidity and mortality.

Managing venous thrombosis in sickle cell disease

Because prospective trials of anticoagulation in VTE have not, to our knowledge, included subjects with SCD, recommendations on VTE management in SCD patients generally rely on clinical guidelines developed for the general population.\textsuperscript{119} It should be noted that, while clinical probability scores and laboratory biomarkers help determine the pretest probability of VTE in the general population,\textsuperscript{120} there is no evidence to support this approach in SCD patients. In addition, the lack of prospective primary prevention and/or management studies of VTE in either pregnant women with SCD or high-risk SCD patients leads to over-reliance on data from prospective studies of VTE in individuals with inherited prothrombotic states.\textsuperscript{39}

Patients with SCD who are suspected of having VTE should undergo compression ultrasound Dopplers for DVT and multidetector computerized tomographic pulmonary angiography and/or radionuclide scanning (ventilation-perfusion [V/Q] scanning) for PE.\textsuperscript{39} Subsequent management of confirmed VTE occurs in two phases: (i) “active treatment” consisting of therapeutic dose anticoagulation for three months to suppress the acute episode of thrombosis; and (ii) “secondary prevention” consisting of therapeutic or prophylactic dose anticoagulation for an indefinite duration to prevent new VTE episodes unrelated to the index event.\textsuperscript{39}

According to ACCP guidelines, in patients with a low risk of bleeding, a risk of recurrent VTE of >13% in the first year results in a strong recommendation and a risk of 8-13% in the first year results in a weak recommendation for indefinite anticoagulation therapy.\textsuperscript{119} Prior cohort studies have suggested that the VTE recurrence rate in SCD ranges from approximately 25-40%,\textsuperscript{8,16} although anticoagulation adherence data are generally lacking in these studies. Nonetheless, the high VTE recurrence rate in SCD patients appears to justify indefinite anticoagulation as an efficacious secondary prevention strategy, especially in patients with unprovoked or recurrent provoked thrombosis.\textsuperscript{121}

Even SCD patients with less severe disease have high VTE recurrence rates (18% at 5 years) with no difference according to whether the incident event occurred within or >90 days after hospitalization,\textsuperscript{6,2} suggesting that most patients are likely to benefit from secondary prevention. However, caution may be warranted, as clinical and plasma biomarkers that reliably predict VTE recurrence in SCD patients have not been identified to guide decision making in this subgroup, unlike in the general population with unprovoked VTE.\textsuperscript{120} When considering indefinite anticoagulation therapy, the perceived risk/benefit ratio influences both patients and physicians alike. Population-based studies reveal a non-linear bleeding risk associated with indefinite anticoagulation, which increases with age, disease comorbidities, polypharmacy, and renal insufficiency.\textsuperscript{21} In addition, a high incidence of bleeding, particularly gastrointestinal bleeding, has been noted in SCD patients exposed to anticoagulation.\textsuperscript{122} Thus, evaluating common risk factors, such as prior bleeding episodes, severe anemia, thrombocytopenia, renal and hepatic failure, use of antiplatelet and NSAID, can inform decisions about both anticoagulant choice and duration of therapy in SCD. Moreover, assessing for central nervous system (CNS) vasculopathy (Moya Moya disease and aneurysmal dilatation of cranial vessels), which can increase the risk of intracranial hemorrhage even in the absence of anticoagulation, is important.

The primary goal of anticoagulation is to limit and resolve thrombosis with minimal perturbation of hemostasis. Among all the available anticoagulants, the DOAC come closest to achieving this goal, although the risk of bleeding is not completely eliminated. For instance, in patients with VTE and no active cancer, a meta-analysis revealed that DOAC were at least as effective as warfarin but reduced the risk of major bleeding only by 40% and did not completely eliminate it.\textsuperscript{123} Prospective evidence from a meta-analysis of two randomized trials of DOAC in cancer-associated VTE reported 6-month outcomes of improved efficacy with DOAC compared to dalteparin (relative risk [RR] of recurrent VTE: 0.65; 95% confidence interval [CI]: 0.42-1.01), but the risk of bleeding was greatly increased (major bleeding RR: 1.74; 95%CI: 1.05-2.88 and non-major bleeding RR: 2.51; 95%CI: 0.85-8.28).\textsuperscript{124} In patients with SCD-associated VTE anticoagulation associated bleeding is particularly evident. Retrospective analysis of anticoagulation for SCD-associated VTE reveals a cumulative bleeding incidence of 4.9% (95%CI: 5.5-6.4%) at 6 months and 7.9% (95%CI: 6.2-9.8%) at 1 year.\textsuperscript{3} In patients with severe SCD, the bleeding risk was greatest (HR: 1.61; 95%CI: 1.11-2.35).\textsuperscript{6} Similarly, smaller retrospective studies indicate that all anticoagulants have an increased bleeding risk, with DOAC having the least risk.\textsuperscript{21,122,124} In spite of these advantages, the exact role for DOAC in the management of VTE in SCD patients is not clear.

Perspectives

Dysregulation of inflammatory and coagulation pathways, both during the steady state and during VOC, are an important hallmark of the sickle prothrombotic state. They appear to be directly associated with the development of thrombotic complications, such as VTE, which is known to increase SCD morbidity and mortality. Given the important role of coagulation in SCD, it is valid to expect that sickle specific therapies seeking to reduce inflammation and coagulation biomarkers would have an effect on lowering thrombosis incidence. Although some sickle specific therapies (HU and transfusion) are associated with reduced arterial thrombosis risk,\textsuperscript{21} surprisingly few studies have evaluated the effect of these therapies.
on venous thrombosis. HU use was associated with reduced biochemical indicators of coagulation activation in SCD patients when compared with non-users, and led to lowered cfDNA levels. In myeloproliferative neoplasms, prospective studies of HU have shown a reduction in thrombosis biomarkers and thrombosis risk, but the lack of similar studies in SCD makes this area a research priority. For instance, targeting P-selectin can diminish heterotypic cellular adhesive interactions (see above) and may prevent vascular endothelial cell activation and injury. Considering its mild adverse event profile crizanlizumab could possibly lower the incidence of thrombosis in SCD without compromising hemostasis.

Given the diverse pathophysiological processes involved in SCD, it is important to narrow down key thrombo-inflammatory pathways involved in VTE pathobiology and design interventions specifically targeting venous thrombosis. From this viewpoint, several trials evaluating the effects on anticoagulants on modulating the vascular pathobiology of SCD are either ongoing or completed (Table 2). However, concerns regarding study design and implementation limit the interpretability of several of these studies, emphasizing the challenges faced by clinical investigation in this field. Nonetheless, implementation of a double-blinded, randomized, placebo controlled trial of prasugrel in children with SCD demonstrates feasibility of rigorous scientific experimentation in this population, and provides hope for future efficacy studies of thrombosis endpoints.

Because traditional anticoagulation for VTE in the general population and in SCD patients is associated with increased bleeding, the development of safer anticoagulant treatments is of considerable importance. Besides, as indicated above, even DOAC fail to suppress TF-mediated inflammation or prevent chronic organ dysfunction in SCD, suggesting the need for novel therapies. Targeting coagulation factors involved in SCD pathobiology (specifically TF and contact pathway components, e.g., FXII and FXI) would appear to have a lowered bleeding risk, especially those that are not involved in physiological hemostasis, i.e., contact pathway factors. For example, anti-XI therapy is both safe and efficacious for VTE prevention in the general population but this approach is untested in SCD. Moreover, as TF plays an important role in physiological hemostasis, therapeutic agents that inhibit TF-mediated inflammation while sparing TF-procoagulant activity are worth developing. Finally, drugs that target post-translational mechanisms regulating TF procoagulant activity, e.g., annexins or thiol-disulfide exchange inhibitors, could prevent pathological thrombosis.

Selecting drugs with a demonstrably lower propensity for bleeding is another approach to maximize anti-thrombotic efficacy in SCD without altering hemostasis. Aspirin, with its low bleeding risk, antithrombotic efficacy for secondary prevention of VTE, and proven safety in SCD may have an important thromboprophylaxis role. Statins reduce markers of hypercoagulability in subjects with unprovoked VTE after cessation of anticoagulation treatment, reduce abnormal pulmonary vascular TF expression in SCD mice, and are generally safe in SCD patients, providing a rationale for their further investigation in VTE thromboprophylaxis. Canakinumab, an IL-1β antagonist, is associated with a reduction in all-cause mortality from atherothrombotic coronary artery disease, largely via its anti-inflammatory effects. Elevated IL-1β levels and dysregulated inflammatory pathways in SCD patients, along with the relative safety of canakinumab in children with SCD (clinicaltrials.gov identifier: NCT02961218), provide a compelling rationale for testing this agent. Evaluating these therapies in the setting of controlled clinical trials would demonstrate their potential role for secondary VTE prevention in SCD.

In spite of the advances in treatment and prevention, venous thrombosis profoundly impacts chronic organ dysfunction and mortality in patients with SCD. Major scientific advances have furthered our understanding of

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**Table 2. Recently conducted or ongoing trials of anticoagulant therapies in sickle cell disease.**

<table>
<thead>
<tr>
<th>Trial identifier</th>
<th>Study design</th>
<th>Intervention</th>
<th>Primary outcome</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT01419977</td>
<td>Phase II randomized parallel assignment</td>
<td>Dalteparin 5,000 IU SC OD x 7 days ex. placebo</td>
<td>Change in value between D1 and D3 of D-dimer, visual analog pain score and thrombin generation tests</td>
<td>Completed 2015 Results awaited</td>
</tr>
<tr>
<td>NCT01036802</td>
<td>Phase II randomized</td>
<td>Warfarin x 12 months ex. placebo</td>
<td>Pulmonary artery systolic pressure measurements on doppler echocardiography</td>
<td>Terminated 2016, due to poor enrollment</td>
</tr>
<tr>
<td>NCT02580773</td>
<td>Phase III randomized parallel assignment</td>
<td>Tinzaparin 175 IU/kg/day x 7 days vs. 4,500 IU/kg/day</td>
<td>Time to ACS resolution and number of major bleeding episodes</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT02179177</td>
<td>Double blind, parallel group, N=25</td>
<td>Apixaban 2.5 mg BID x 6 months ex. placebo</td>
<td>Effects on hospitalization days, daily pain scores, and pain crisis frequency</td>
<td>Terminated 2019, due to lack of funds</td>
</tr>
<tr>
<td>NCT02072668</td>
<td>Phase II randomized cross over with 2 weeks wash out period N=14</td>
<td>Rivaroxaban 20 mg OD x 4 weeks ex. placebo</td>
<td>Biomarkers of inflammation, endothelial activation and thrombin generation; microvascular flow</td>
<td>Completed 2019 Results awaited</td>
</tr>
<tr>
<td>NCT02098993</td>
<td>Phase II randomized pilot feasibility study N=7**</td>
<td>Unfractionated heparin therapeutic dose ex. standard care</td>
<td>Duration of hospitalization for acute chest syndrome</td>
<td>Terminated 2019, due to poor enrollment</td>
</tr>
</tbody>
</table>

SC: subcutaneous; OD: once a day; D: day; ACS: acute chest syndrome. *N=16 recruited at study termination. **Actual enrollment prior to termination.
the vascular pathology of SCD and led to the development of novel therapeutic agents optimal for clinical investigation. Conducting effective preventative and treatment studies to reduce VTE in SCD and establishing the evidence base to guide appropriate management has therefore become a research priority. The design of these studies should include endpoints that account for simultaneous effects on anti-thrombotic efficacy and harm due to bleeding.

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