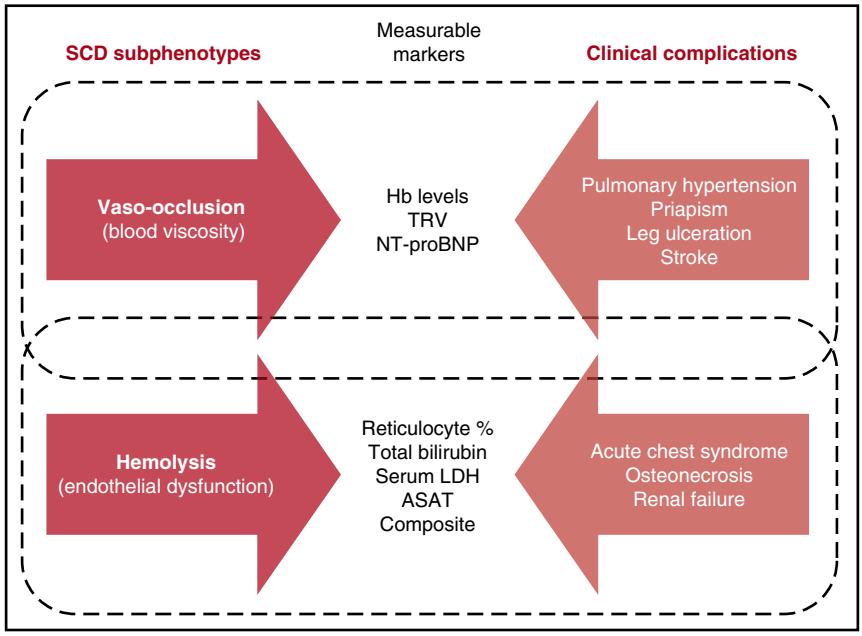

Subphenotypes of SCD, measurable markers, and clinical complications. ASAT, aspartate aminotransferase; Hb, hemoglobin; LDH, lactate dehydrogenase; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; TRV, tricuspid regurgitant jet velocity. Adapted from Figure 2 in Kato et al.[7]

Nevertheless, the authors set out to investigate the HHP with an open mind and found only limited support for a clear phenotypic divide based on the assessment of hemolysis alone. This study illustrates some of the real difficulties associated with the collection of rich phenotype data under challenging circumstances and the remarkable levels of morbidity that are seen in Africa, particularly within adult populations.

Beyond the HHP controversy, Dubert et al's study also documents 2 additional challenges concerning the prevention and treatment of SCD in Africa. First, the authors provide clear evidence for significant differences between some of the different genotypes of SCD, including SCA, sickle-β^0 thalassemia, sickle-β^+ thalassemia, and hemoglobin SCD in terms of baseline characteristics and clinical complications. Although this observation is not in itself novel, the size of this study reinforces the potential need for the tailored treatment of different SCD subgroups. Second, this study highlights the need for reliable easy-to-use markers of anemia and hemolysis in LMICs. The etiology of anemia in African patients with SCD may be very different from that in resource-rich countries, potentially including additional factors such as malnutrition (eg, iron deficiency) and infectious diseases (eg, malaria and helminth infestations). Accurately quantifying hemolysis requires direct measurements of the lifespan of red blood cells. Most studies published in relation to the HHP so far, including this one, have used indirect markers such as reticulocyte counts, aspartate aminotransferase, total bilirubin, and lactate dehydrogenase levels. Technological advances similar to those seen in recent years in the development of point-of-care testing devices[8] would be a valuable asset to this research agenda going forward.

Assuming that financial and logistical challenges can be overcome, the sheer number of patients affected by SCD in both sub-Saharan Africa and India offers a huge opportunity to further our understanding of the epidemiology and pathophysiology of this important condition. Better data will help us to define the optimum approaches to prevention and management policies in LMICs and reduce the global burden of SCD. Further work of the type described in this article will help us to piece together the pathophysiology of SCD for the benefits of all patients with this condition, wherever they may live.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● THROMBOSIS AND HEMOSTASIS

Comment on Haining et al, page 2224

Platelet CLEC-2: a molecule with 2 faces

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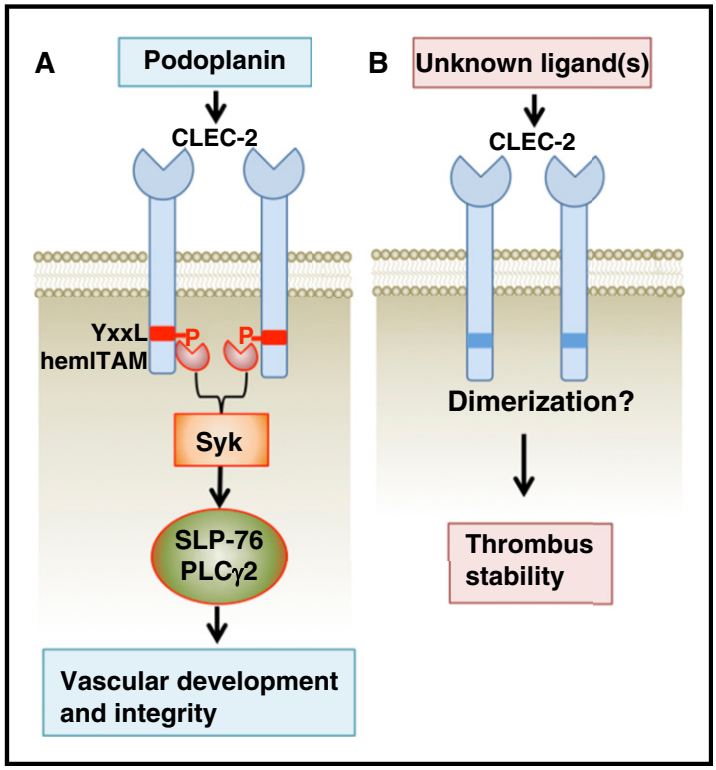
In this issue of *Blood*, Haining et al reveal an unexpected role for platelet C-type lectin-like receptor-2 (CLEC-2) in thrombosis, which is independent of its hemi-immunoreceptor tyrosine-based activation motif (hemITAM) signaling.[1]

CLEC-2 is a highly expressed platelet receptor.[1,2] Its intracellular domain contains a unique single YxxL motif (hemITAM) that is capable of activating platelets through downstream signaling effectors Syk and SLP-76 upon ligand



Model showing a hemITAM-dependent function of platelet CLEC-2 in vascular development and integrity (A), as well as a hemITAM-independent role of platelet CLEC-2 in thrombus formation (B). P, phosphorylated.

engagement of a CLEC-2 dimer. Platelet CLEC-2 is involved in 2 important biological processes: blood/lymphatic vessel separation and thrombosis. The former function has been well characterized in mice lacking platelet CLEC-2 or its intracellular signaling molecules Syk or SLP-76 because these mutant mouse models share a distinct blood-lymphatic mixing vascular phenotype. This indicates a critical function of hemITAM signaling for CLEC-2: to regulate blood-lymphatic separation during lymphatic vascular development.[3] The blood-lymphatic mixing phenotype is also seen in mice lacking the O-glycoprotein podoplanin,[4] the only known physiological ligand of CLEC-2. Podoplanin is specifically expressed on lymphatic endothelial cells. Therefore, these data indicate that platelet activation mediated by CLEC-2 hemITAM signaling upon binding to lymphatic endothelial podoplanin is essential for forming an independent lymphatic vascular system. Other than this well-defined role in vascular development, mice with CLEC-2–deficient platelets also exhibit impaired platelet aggregate formation and lower susceptibility to arterial thrombus formation relative to wild-type controls.[5,6] Podoplanin does not seem to be involved in platelet CLEC-2–mediated thrombus formation as it is expressed neither on blood endothelial cells nor on other blood cells under physiological conditions.[7] But, whether hemITAM-mediated signaling is required in this biological process remains to be determined.

In this study, Haining et al generated a novel knock-in mouse model that expresses a mutant form of CLEC-2 with its cytoplasmic hemITAM YxxL motif mutated (Y7A KI). The Y7A KI platelets express normal surface levels of CLEC-2 as well as other platelet surface receptors. Y7A KI platelets display normal responses to classic agonists such as thrombin and collagen, but have a defective response to the CLEC-2 agonist rhodocytin. By binding to CLEC-2, both rhodocytin and podoplanin activate platelets through hemITAM-dependent signaling. Like CLEC-2 knockout (KO) mice, Y7A KI mice have the blood-lymphatic mixing phenotype, confirming the critical role of the hemITAM-dependent signaling of CLEC-2 in promoting separation of lymphatic vessels from the blood vessel during development. However, unlike CLEC-2 KO mice, Y7A KI mice have normal bleeding time and thrombus formation, in 2 different arterial thrombosis models, similar to that of wild-type controls. Importantly, treatment of Y7A KI mice with the Fab′ fragments of the anti-CLEC-2 antibody, INU1, which does not cross-link CLEC-2 to induce hemITAM signaling, reduces thrombus formation, revealing a hemITAM signaling–independent role for CLEC-2 in thrombosis.

Haining et al show that CLEC-2 is required for platelets to interact with other platelets adherent to sites of vascular injury within the vessel to form thrombus, which again raises the often-asked question: how does platelet CLEC-2 contribute to thrombus formation (see figure)? Podoplanin is not known to be present on platelets or other blood cells under normal conditions.[7] In addition, the INU1 Fab′ fragments block aggregate formation of Y7A KI platelets on collagen under flow conditions in vitro. These data are suggestive of additional endogenous ligand(s) for platelet CLEC-2. There have been several attempts to identify new ligands/counterreceptors for platelet CLEC-2, but none of the studies appear to be conclusive. Thus, future studies are required to reveal the mysterious blood-borne CLEC-2–interacting molecule(s) and its binding site on CLEC-2. Podoplanin and CLEC-2–mediated platelet activation is also critical for protecting vascular integrity in many important organs, such as lymph nodes and the brain.[7,8] This hemITAM signaling–independent platelet CLEC-2 function is neither required for vascular development/integrity nor involved in hemostasis, that is, initial platelet adhesion. Therefore, it represents an attractive therapeutic target for development of an effective and safe antithrombosis therapy.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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