Sickle cell disease severity: an introduction

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This thematic issue of *Experimental Biology and Medicine* is dedicated to the subject of advances in our understanding of clinical and genetic factors that impact Sickle Cell Disease (SCD) severity. The contributing authors are leaders in the field covering topics including models to define sickle cell severity; the molecular and cellular bases of sickle cell severity; clinical trials related to treatment development for SCD and future precision medicine, based on biomarkers which can predict SCD severity. We will begin by introducing the subject of sickle cell severity and then move on to the individual articles in this special issue.

Sickle Cell Anemia (SCA) is the most prevalent form of SCD in which the disorder arises from homozygous single nucleotide polymorphisms (SNP) in the sixth codon of the β -globin gene (HbSS) on chromosome 11. This leads to hemoglobin molecules that form polymers under deoxygenated conditions which in turn causes the red blood cell (RBC) to convert from a biconcave to a sickled shape. The sickled RBC also becomes dehydrated due to K^+ leakage and water loss, and has a shortened lifespan. This dehydrated sickled RBC slows the blood flow and mediates the association of leukocytes with the blood vessel endothelial wall, causing microvascular obstruction referred to as vaso-occlusion and leading to painful sickle cell crises. When blood flow is reestablished this promotes ischemia reperfusion injury and inflammation. While SCD is a monogenic hemoglobinopathy the clinical severity experienced by each patient varies widely, as does their lifespan, with a correlation between the number of vaso-occlusive crises per year and survival. The pathology associated with SCD begins within the first year of life, to varying extent based on the clinical severity of each individual. An ability to know who will have a mild, moderate or severe phenotype would allow precise individualized treatment of infants and young children. The only FDA-approved drug for SCA is hydroxyurea (HU), with other drug therapeutics in the pipeline, and bone marrow transplantation is curative.

To set the tone for this thematic issue, Quinn's minireview¹ explains the individual variability in specific complications of SCD. Each of these disease severity manifestations can be measured, but the difficulty in modeling global sickle cell severity in this multi-organ disease is

discussed. Quinn goes on to explain the determinants of sickle cell severity with fetal hemoglobin (HbF) content and distribution being the most potent. HbF acts by inhibiting the polymerization of HbS under deoxygenated conditions in post-capillary venules. Other determinants are co-inherited such as α thalassemia; glucose-6-phosphate dehydrogenase deficiency; UGT1A1 promoter polymorphisms; and high leukocyte counts. Habara and Steinberg's minireview² discusses genome-wide association studies (GWAS) that have been used to discover genes or intergenic regions that impact HbF content, bilirubin concentration, stroke, vaso-occlusive crisis rate, acute chest syndrome (ACS) rate, infection, osteonecrosis, priapism, leg ulcers, tricuspid regurgitation velocity, cholelithiasis, renal function and hemolysis in SCD.

The importance of HbF in protecting against severe clinical manifestations of SCD was demonstrated by the mild clinical course of individuals who are compound heterozygous for HbS and hereditary persistence of fetal hemoglobin (HPFH). In primary research articles related to HbF expression Braghini et al.³ produced β -YAC transgenic mouse models which recapitulate HPFH to establish a valuable *in vivo* model for investigating γ -globin gene regulation. Liu et al.⁴ performed a case-controlled GWAS study using a novel Illumina Omni1-Quad chip containing 1,140,419 high-density SNPs with 76% coverage of SNPs validated in people of African ancestry. Comparing subjects with high and low HbF they identified novel SNPs in the second intron of *BCL11A* and four previously unreported candidate genes (*SPARC*, *GJC1*, *EFTUD2*, and *JAZF1*) related to HbF expression. To expand the regulatory molecules that control globin gene expression work was conducted to discover non-coding micro-RNA molecules. Ward et al.⁵ demonstrated for the first time in K562 cells, that microRNA-34a increase HbF levels by a mechanism involving silencing of the negative regulator STAT3, which binds in the γ -globin 5' untranslated region.

Hydroxyurea is the only current FDA-approved drug to treat SCD, mainly through its ability to induce HbF. Wang's minireview⁶ discusses the adult and pediatric clinical trials which have led to its approval and the recent NHLBI recommendation that HU treatment be offered to children with

SCA starting at nine months after birth regardless of clinical symptoms. Clinical studies in the thematic issue include a retrospective study by Summarell and Sheehan⁷ on the use of HU in 14 pediatric patients with HbSC disease, a group which has been excluded in prior HU trials. They found that five of the 11 patients, who reached maximum tolerated dose, failed to show clinical improvement. Four of the five were then placed on dual HU and phlebotomy treatment, which produced clinical improvement indicated by improvement of severe retinopathy, or reduction in chronic opioid use. Pahl and Mullen⁸ performed a retrospective study on SCD patients receiving care at the University of Rochester. They found that ACS was most common in patients with HbSS and HbS β^0 -Thalassemia genotypes and diagnosed with asthma; validating prior studies. Furthermore they observed poor documentation of ACS and asthma in the electronic medical records and a lack of consultation from pediatric pulmonary specialists. Hankins et al.⁹ have performed a retrospective study on 330 SCD subjects to determine the risks and benefits of HU in 120 subjects diagnosed with aplastic crisis due to Parvovirus B19 infection. Those patients on HU required fewer transfusions and had a higher hemoglobin concentration nadir. Interestingly, treatment with HU had no effect on the ability of children to generate protective Parvovirus B19 immune responses.

To gain expanded knowledge about blood flow, innovative studies were conducted by Rivera et al.¹⁰ using computational fluid dynamics to perform two and three dimensional simulations to investigate whether high blood velocities confer greater stroke risk in children with SCD. They provided insight into how specific vascular geometry can cause local flow disturbances that may lead to increased blood velocities in SCD. Tang et al.¹¹ demonstrated, utilizing the Townes transgenic SCD mouse model, that hypoxia promotes ischemic tissue injury while increasing hemolysis scavenger molecules such as haptoglobin and hemopexin. This duality may provide insight into human clinical symptoms associated with vaso-occlusion and hemolysis.

Many different aspects of sickle cell severity have been reviewed and investigated in this special thematic issue. To put these studies into context, Goodman et al.¹² contributed a mini-review which discusses candidate bio-markers which have been identified to-date by multiple-omics approaches (genomics, transcriptomics, microRNAomics, proteomics, metabolomics and integrated interactomics).

They suggest that multiomic, or omic stack approaches, coupled to big data analyses, will ultimately lead to powerful individual and panels of verified and then validated biomarkers for sickle cell severity. Subsequently, these validated biomarkers can be used in longitudinal clinical studies to determine which will be prognostic early in life for predicting sickle cell severity. This in turn will allow precision medicine for SCD where prognostic biomarkers will make possible individualized treatment.

The Editors of this issue hope that it will be of value to both experts in the field of SCD research as well as those *Experimental Biology and Medicine* readers who wish to learn about this subject.

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