Invited Article

Perspective: A Novel Prognostic for Sickle Cell Disease

Brian M. Mozeleski, Abdullah Al-Rubaish[1], Amein Al-Ali[2], Jose Romero[3]

MyoSyntax Corporation, Worcester, Massachusetts, USA, Departments of [1]Internal Medicine and [2]Biochemistry, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Kingdom of Saudi Arabia, [3]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA

Abstract

Sickle hemoglobin ($\alpha_2\beta^S{}_2$) polymerization drives disease pathophysiology in sickle cell anemia. Fetal hemoglobin ($\alpha_2\gamma_2$) restricts disease severity by inhibiting the polymerization of sickle hemoglobin in a concentration-dependent manner. Clinical decision-making relies on diagnostic technologies evaluating fetal hemoglobin as mean percent or mean quantity in blood. Limitation of this approach is exemplified by patients with significant high fetal hemoglobin levels and severe disease, suggesting that fetal hemoglobin is unevenly distributed across F-cells. Therefore, determination of fetal hemoglobin/F-cell would provide a new paradigm for ascertaining prognosis and response to fetal hemoglobin-inducing agents. Measurement of fetal hemoglobin/F-cell, ultimately adapted to widespread standardized analytical use, is a promising fetal hemoglobin-related prognostic approach to monitor the severity of sickle cell disease and the best "phenotype" to follow when developing new candidate fetal hemoglobin inducers or titrating hydroxyurea in treated sickle cell patients.

Keywords: Fetal hemoglobin, sickle cell disease, sickle hemoglobin

Address for correspondence: Dr. Brian M. Mozeleski, MyoSyntax Corporation, 60 Prescott St, Worcester, MA 01605, USA.
E-mail: bmozeleski@myosyntax.com

INTRODUCTION

Sickle cell disease is a major clinical problem worldwide, expected to increase by about 30% globally within the next 30 years.[1] It is highly prevalent in Sub-Saharan Africa, India, the Kingdom of Saudi Arabia and the United States. Its complications are legion and death is premature. According to estimates, approximately 100,000 Americans suffer from sickle cell disease, and worldwide, about 300,000 children are born with this disorder each year.[1,2] Further, the economic impact of sickle cell disease is immense; for example, in the United States alone, the estimated annual cost of medical care exceeds USD 1.1 billion.[3] Sickle hemoglobin (HbS, $\alpha_2\beta^S{}_2$) polymerization drives the pathophysiology of this disease, while fetal hemoglobin (HbF, $\alpha_2\gamma_2$) inhibits the deoxygenation-induced polymerization of HbS.[4,5] HbF is present throughout fetal development, but is mostly replaced by adult hemoglobin (HbA, $\alpha_2\beta_2$) around 6 months after birth.[6,7] A major therapeutic goal in sickle cell disease management is to induce high HbF levels in sickle erythrocytes.[8-10]

FETAL HEMOGLOBIN AND SICKLE CELL DISEASE

HbF reduces HbS concentration, but more importantly, neither HbF nor its mixed hybrid tetramer ($\alpha_2\beta^S\gamma$) can enter the deoxy-HbS polymer phase.[11] At birth, HbF accounts for approximately 85% of total hemoglobin; however, after 1 year, HbF level falls to <1% of total hemoglobin,

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with an inverse increase in HbA.[9] This process is known as hemoglobin switching.[12] However, in patients with sickle cell disease, the switch from HbF to HbS is delayed, and HbF does not reach stable levels until the age of 5–10 years.[13,14] When segregated based on β-globin gene (HBB) haplotypes, a small proportion of patients have HbF levels of 10%–20%, but most have a mean HbF of 5%–8%.[8,10,15,16] HbF concentration and its distribution in erythrocytes are heritable and regulated by elements that are *cis* and *trans* to the γ-globin genes, thereby contributing to the wide range of HbF levels among patients.[10,12] This variability in HbF is associated with significant clinical differences among large groups of patients across these haplotypes. Nevertheless, all patients are anemic and most are symptomatic.[10] The heterogeneity of symptoms among patients may be a result of the different levels of HbF or its progeny F-cells,[8,17] as more evenly distributed HbF levels among erythrocytes leads to amelioration of symptoms and nearly normal hemoglobin levels.[10,12,17]

FETAL HEMOGLOBIN VARIANCE ACROSS HAPLOTYPES

The four major haplotypes (Bantu, Benin, Senegal and Arab-Indian [AI]) differ in HbF concentrations. HbF concentrations are highest in AI haplotypes, yet they lack full protection, as clinical symptoms are frequent. Saudis with the Benin haplotype (half the HbF concentration of AI haplotypes) have an HbF level of 15%–20% and experience symptoms at a similar frequency as AI haplotype.[14,18] While mean expression data have been widely characterized, a haplotype-specific distribution of HbF/F-cell has not yet been published. Assessment across the respective haplotype diversity will be crucial in achieving sufficient statistical power, and thus precise prediction of disease severity.

F-CELLS AND THE CELLULAR DISTRIBUTION OF FETAL HEMOGLOBIN

HbF is produced in some erythroid progenitor cells, and its concentration and distribution in erythrocytes are heritable.[7] HbF can be quantified by high-performance liquid chromatography (HPLC), which is highly correlated with F-cells ($R^2 = 0.97$).[19,20] F-cells comprise both HbF and HbA. In individuals without the sickle trait, F-cells, when measured by a sensitive cell-based method, contain ~4–5 pg of HbF per cell with limited distribution range.[21] Sickle cell disease patients, with an average HbF/F-cell quantity of 6.4 ± 1.6 pg, display large F-cell variation ranging from 2% to 80%.[21] Compound heterozygotes for HbS and gene deletion hereditary persistence of fetal hemoglobin (HPFH) are neither symptomatic nor anemic and contain ~10 pg HbF per cell, which is evenly distributed. These observations drove the understanding of HbF/cell protection levels of 10 pg HbF/cell with ~30% of HbF.[22] While the origins, genetics and physiology of F-cells have been well described,[4,6] the vast range of unique HbF/F-cell distribution profiles, irrespective of the total HbF level,[23] serves as an intriguing prospect for future study. This issue becomes more relevant as we improve our understanding of the genetics controlling HbF levels.[24] Investigators should exercise caution when selecting assay methodology, as limits of detection fail to capture the full distribution of F-cells. HPLC is the *in-vitro* diagnostic gold standard for qualitative screening of hemoglobins (F, A, S, D, C and E), expressed as a percent of total hemoglobin. HPLC using the Bio-Rad Variant™ Sickle Cell Short Program received the US Food and Drug Administration approval in 1993 [510(k) Number: K051072] and remains the preferred choice to date. Imaging flow cytometry (IFC) has provided an opportunity for quantitative measure of HbF per cell while simultaneously capturing cellular characteristics such as size and morphology. However, IFC is likely to mainly remain a research tool, as acquisition is often timely and unlikely to meet turnaround requirements for diagnostic devices. Standard flow cytometry, for example, offers a detection threshold of ~6 pg HbF/cell with well-characterized clones.[9,25,26] As HbF diagnostics have relied on mean HbF levels and % F-cells, which do not account for HbF level variance across the F-cell population,[27] we hope that the promise of HbF/F-cell distributions methodology yields fruitful results.

THE MOTIVATING RATIONALE

One of the most promising methods for pharmacological treatment of sickle cell disease is the therapeutic induction of high levels of HbF.[8,9,19] These methods induce HbF levels by targeting the proximal pathophysiological driver of sickle cell disease. Hydroxyurea (HU), the principal drug agent for sickle cell disease therapy, is well-documented in its proficiency to induce high levels of HbF across a range of sickle cell haplotypes, and thus has resulted in significant clinical benefits among many patients.[28,29] Conversely, patients treated with HU can have sudden life-threatening complications, including those whose treatment results in HbF of ≥20%.[30,31] Following treatment administration, most patients have persistent, although reduced, vaso-occlusive and hemolytic crisis extending beyond the initial few years of life. It has been speculated that early-life HU administration potentially decelerates the age-related decline in HbF. After 10 years of age, HbF levels can gradually decline and are often

accompanied by worsening symptoms, despite HbF levels of ~20%, as many F-cells remain poorly "protected" from polymer-induced damage.[9] This has driven the pursuit for alternate HbF-induction therapies. In transgenic sickle mice, inactivation of BCL11A nullified the complications of the disease.[32] It should be noted that these animals have homogeneous distributions of HbF, similar to HbS–HPFH, where pancellular distribution of HbF was adequate to prevent polymer-induced damage.[32] It is yet unknown if targeting BCL11A or its pathway in humans will lead to a pancellular or heterocellular distribution of HbF in erythrocytes.[33] Therefore, development of a novel diagnostic tool, after clinical evaluation, would provide investigators and clinicians increased prognostic guidance and potentially accelerate development of novel therapeutic strategies such as gene therapy, novel HbF inducers and adaptive dose of HU.

DISTRIBUTION VERSUS THE MEAN: ACHIEVING PROGNOSTIC PRECISION

The approach to increase prognostic precision by assessing the distribution of absolute HbF quantity across single F-cells (versus mean) is analogous to precision medicine adapted to individuals versus population stratification. Normal ranges are currently being redefined to specific populational subsets, providing clinicians with increased insights that have vastly improved clinical outcomes. The introduction of precision profiling techniques, such as absolute HbF/F-cell quantification, hold the potential to quantify responsiveness and precise prognostic correlation. Survival increases when HbF concentration exceeds ~8% of total hemoglobin.[17] At the population level, qualitative HbF levels are associated with reduced clinical presentation, while at the individual level, HbF levels offer limited prognostic precision. Contrasting the distribution of sickle cell anemia patients with high levels of HbF and high disease severity will be a fascinating revelation of the requirements for protection. As HbF/F-cells are highly variable and clinically relevant, the predictive value of HbF would be improved with an assay that determines both HbF concentration and distribution within F-cells.

PROGNOSTIC IMPLICATIONS FOR THERAPY

HU administration is currently the best available treatment for sickle cell anemia. HU induces HbF, reducing disease morbidity and mortality, but does not cure the disease. HU treatment response is highly variable with differential induction of HbF levels across responders (mean HbF/F-cell is ~8 pg) and a significant proportion of nonresponders.[19] Even patients with ~20% HbF have been described to have life-threatening conditions. Despite HU being a potent inducer of HbF, its failure to provide comprehensive protection drives speculation that to achieve its benefit, combinatorial drug targeting of HbF induction with drugs altering erythropoiesis mechanisms could provide improved therapeutic efficacy. For example, if erythropoiesis-stimulating drugs altered HbF distribution, patients with high HbF levels and high disease severity may achieve protection, albeit with a different distribution of HbF in F-cells.

CONCLUSION

We believe that analysis of HbF/F-cell distribution profiles offers the greatest promise to improve severity of sickle cell disease prognosis. As we draw closer to validating a method for the determination of HbF/F-cell across a diversity of haplotypes, we hope its utility serves as a platform to improve the welfare of individuals who suffer from sickle cell disease.

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Conflicts of interest

There are no conflicts of interest.

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