HealthTech Report

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Sickle cell disease/anemia:

Review of needs across the life span and opportunities for innovative technology solutions

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Introduction

Sickle cell disease (SCD), also referred to as sickle cell disorder and sickle cell anemia, is a group of inherited blood disorders consisting of the most common gene disorders found worldwide.[1] SCD is estimated to be found in 5% to 7% of the world's population,[1,2] with 200,000 to 300,000 infants born with the disease every year worldwide.[3,4] About 80% of affected children are born in developing countries,[5] three-quarters of whom are in low-income regions of sub-Saharan Africa.[2] In Africa alone, this translates into nearly 700 children born with SCD every day,[2] with an estimated 16% of the population in Africa affected by the disease.[6] India also bears a substantial proportion of burden; over 44,000 newborns were born with SCD in India in 2010, which accounted for approximately 13% to 20% of global cases that year.[7] SCD has a significant impact on mortality, with only an estimated 25% of babies born with SCD annually living to their fifth birthday.[3] As measured by under-five mortality, SCD contributes the equivalent of 5% of under-five deaths on the African continent with more than 9% of such deaths in West Africa and up to 16% of under-five deaths in individual West African countries.[3] Adults with SCD in high-resource countries, including the United States, have decreased life expectancy—they have a less than 30% chance of surviving beyond the seventh decade of life.[8]

Description

Normal red blood cells (RBCs) are disc shaped. This shape enables normal blood cells to move easily through blood vessels. One function of RBCs is carried out by the iron-rich protein hemoglobin which carries oxygen from the lungs to the rest of the body. Sickle cells contain abnormal hemoglobin called sickle hemoglobin or hemoglobin S, which causes cells to develop a sickle or crescent shape. Sickling causes cells to become stiff and sticky with decreased flexibility.[9] This leads to the tendency of sickled cells to block blood flow, causing acute painful episodes and increasing the risk of organ damage as well as infection.[10]

Pathogenesis

SCD is caused by a genetic mutation that causes the 6th amino acid in the B-globin chain to substitute valine for glutamic acid and adenine for thymine at the 17th nucleotide.[4,11] There are many types of SCDs, as this term is used to refer to the condition where there is a sickle cell gene together with any other abnormal B-globin gene.[6]

The phrase "sickle cell anemia" or SCA is generally used to refer to homozygosity for the β^s allele and individuals having two alleles for the mutation. Therefore, SCA is also referred to as "HbSS," "SS disease," "hemoglobin S," or permutations of those names.

In heterozygous individuals—those who have only one sickle gene and one normal hemoglobin gene—the condition is referred to as "HbAS," "sickle cell trait" (SCT), or "carriers." The prevalence of sickle cell gene carriers varies between 5% and 40% of the population.[12] Carriers of SCT have an increased resistance to malaria which has led the trait to becoming widespread among populations of malaria-endemic or previously endemic countries.[11]

SCD is widespread in sub-Saharan Africa, the Mediterranean, South and Central America, the Middle East, and central India. With migration, the condition has become a global disease; as noted previously, SCD is the most common genetic disease in the United States.[13]

Rarer forms of SCD include conditions where an individual has one HbS gene and another abnormal hemoglobin allele (referred to as compound heterozygous). These include sickle-hemoglobin C disease (HbSC), sickle beta-plus-thalassemia (HbS/β^+), and sickle beta-zero-thalassemia (HbS/β^0).[9] In addition to many types of different HbS/β-thalassemia, there are at least ten other genotypes that cause SCD. Of children born with a disorder, roughly 70% have SCD/SCA, and the remaining have thalassemia and other forms. Most of these other SCD gene variants are rare, and many are harmless. However, 50,000 to 100,000 children with thalassemia major die each year in low- and middle-income countries from complications related to the condition.[5] Currently there is less evidence to guide management of these other types of SCD.[11]

In 2005, SCD was recognized as a public health priority by the United Nations Education, Scientific, and Cultural Organization and by the African Union, followed by the World Health Organization (WHO) in 2006.[12] WHO has declared that screening and genetic counseling for hemoglobin disorders should be incorporated into regular health care in most countries.[3]

Presentation and course

Identifying children at birth is critically important because children with SCD are most susceptible to complications during the first five years of life. Sickle cells can obstruct blood flow to the spleen resulting in functional asplenia (lack of normal spleen function), which in turn causes susceptibility to life-threatening infections.[14,15] Because of this the first presentation of the disease is potentially catastrophic.[1,16] In all ages, painful vaso-occlusive episodes (VOEs) are a hallmark of SCD. These result in recurrent painful events and also a variety of serious organ system complications, which often lead to disability, suffering, and/or early death.[6] In young as well as older children and adults, the most common causes of morbidity and mortality in SCD are infection, neurological complications, acute coronary (or chest) syndrome, splenic sequestration, pulmonary emboli/thrombi, pulmonary hypertension, renal failure, hepatic failure, massive hemolysis, and heart disease, specifically left ventricular failure.[17,8,11] SCD carriers also suffer from complications and are at increased risk for additional conditions; there is even some evidence for sudden, unexplained death.[18]

Prevention

In couples where both partners are carriers, with each pregnancy there is a 25% chance of having a child with SCA, a 50% chance of having a child with SCT, and a 25% chance of having a child with normal hemoglobin.[13] Genetic testing of adults and/or prenatal screening can offer a couple choices at pre-conception or early in pregnancy.

In high-resource areas, detecting those with SCD as well as carriers and informing them of their risk and possibilities for reducing it has led to a decrease in births and deaths of affected

children.[19] This occurs because at-risk couples limit their family size to fewer children, there is high uptake of prenatal diagnosis, and some carriers avoid risk by selecting a non-carrier partner. The screening and information mechanisms found effective are: (1) retrospectively informing parents with affected children of their 25% recurrence risk, (2) prenatal diagnosis for couples, and (3) carrier screening for the whole population. Offering testing in high school or before marriage allows a wide range of choices and requires the least number of laboratory tests.

Currently, genetic and prenatal testing are not routinely offered in low-resource settings (LRS). The lack of screening tests that are more appropriate for these settings presents challenges and opportunities. Ideal testing would be noninvasive and accurate and be able to be combined with other screening for common genetic disorders.[20,21] Other important characteristics of tests suitable for LRS include minimal requirements for equipment, reagents, power, running water, or cold chain; minimal need for training; portability; short turnaround time for results; extended shelf-life; and stability at varying temperatures.[22]

Early detection

The standard of care in developed countries includes newborn screening (NBS) followed by prophylaxis and comprehensive care. In developed countries, these interventions have been successful in improving outcomes and decreasing mortality due to SCD among children younger than five years.[1,23] NBS has also been successfully piloted in a number of African countries, and some are in the process of adopting it as their standard of care.[24]

Current standard-of-care diagnostic tests for SCD in developed countries are based on analysis of hemoglobin.[11] These include hemoglobin electrophoresis, iso-electric focusing, and high-performance liquid chromatography (HPLC). Abnormal screening results are usually retested with a complementary electrophoresis technique, HPLC, immunologic tests, or DNA-based assay.[15] These tests are highly accurate, can be used on dried-blood-spot samples (appropriate for LRS), and differentiate between SCD and SCT. However, because they involve a clinical lab with specialized equipment, highly trained technicians, and lengthy processes, these tests are expensive, largely unavailable, and unsuitable for point-of-care use in low-resource countries.[25] Automated instruments, mass spectrometry, and DNA analysis have been developed to increase throughput and decrease processing time in high-resource countries,[11] but again due to cost, these are currently unavailable to LRS.

Diagnosis of SCD at the point of care (POC) is ideal in order to initiate critical therapies and to prevent loss to follow-up during the postnatal period. A POC test currently in use for SCD is *SICKLEDEX*® (Streck) which is commonly used by blood centers for routine screening of donated blood. The test can easily be used at the POC because it has a relatively fast (5–15 minutes) processing time, is inexpensive, and has minimal requirement for reagents. However, it only detects the presence of HbS and does not differentiate between SCD and SCT. It also has relatively poor diagnostic accuracy (sensitivity of 45% and a positive predictive value of 33.3%)[26]— acceptable for a blood screening tool but not for a diagnostic test. It cannot detect HbS in persons with severe anemia or in infants younger than six months of age due to elevated levels of hemoglobin F. In addition, the shelf-life for the buffer is relatively short.[25]

Another tool for initial screening is direct microscopic observation using a peripheral blood smear preparation and deoxygenating agent. Low equipment needs (microscope, reagents) allow it to be easily implemented in LRS, particularly because other diagnostics common in these settings use the same method (e.g., malaria smears). However, it may not be a very sensitive technique in newborns due to the high percentage of fetal hemoglobin (HgF) present until approximately six months of age. In addition, this method is heavily reliant on human interpretation and thus subject to human error.

The lack of affordable and effective SCD POC diagnostics presents a clear need and opportunity for innovation. Accessible screening is key to initiating life-saving preventive and therapeutic measures. In LRS, such tools would enable health facilities to offer SCD screening locally, decreasing the burden on overcrowded central hospitals and laboratories and decreasing wait times.[25] Chen, et al. also make the case that in developed countries a faster diagnostic tool could decrease barriers to treatment.[4,25] Due to long wait times for laboratory results, diagnosis of SCD at the POC is often done through elimination of other possible illnesses, which is inefficient and may result in delay of treatment and unnecessary suffering.

Preferred capabilities and characteristics of a test include ability to differentiate between carriers and SCD, is highly accurate and can diagnosis newborns while HgF is still raised (up to six months of age), and delivers results in a timely manner.

Recent publications have reported on the development of novel tests that have potential to meet these requirements. Yang, et al. describe a paper-based test that assays hemoglobin solubility; it is simple, inexpensive, and can diagnose SCD definitively. [27,28] Another assay innovation based on hemoglobin solubility is called the sickle confirm modified hemoglobin solubility test.[29] Additionally, another group is working on a non-electrolyte hemolysis assay based on the reversible increase in red cell membrane permeability generated by hemoglobin S polymers in the deoxygenated state. This permeability, referred to as P (sickle), triggers a chain of events in which two constitutive transporters of the red cell membrane become activated—causing the RBCs of patients with SCD to lyse. This has been proposed as a novel and sensitive way to assay for SCD and even different types of SCT; however the dependence of the lysis on oxygenation of the lysis solution, temperature, and pH makes its robustness in the relatively uncontrolled environments of LRS laboratories difficult to predict.[30]

Some of these tests do not work well for screening infants (less than one year) due to high levels of HgF.[15] A technology that has not yet received much attention for LRS and should be explored is an antibody-based test.[31] Sensitive monoclonal antibodies specific for either Hb A, Hb F, Hb S or Hb C have been used for enzyme-linked immunosorbant assays (ELISA) and as single-step immune labels for flow cytometric analysis to monitor hydroxyurea therapy.[32] Radial immunodiffusion (RID) assays have also been investigated, although with less success.[33] Flow cytometry is too complex and expensive for LRS, and ELISA and RID are demanding enough that they are confined to better-equipped and -staffed hospital laboratories in LRS. However, lateral flow strips (LFS) to detect antigenic biomarkers for infectious diseases are common in LRS due to their simplicity and ease of use. The technical challenges in adapting existing sensitive and specific antibodies to the LFS format should be manageable, and compared to most infectious diseases the quantity of analyte in a typical blood sample is quite large—a finger-stick sample should suffice without demanding great analytical sensitivity from the assay. Program

officers at the National Institutes of Health seem to agree, as a recent funding opportunity suggests.[i] LFS are not generally quantitative unless a reader instrument is used along with them; however, a few LRS-appropriate POC readers are available (e.g., the Becton-Dickenson Veritor system[34]), and a partnership could be explored. Alternatively, better availability of ELISAs could address the need for a quantitative test. Semi-quantitation can be done visually and might be sufficient for screening purposes. If the typical monoclonal antibody production techniques were unable to yield the appropriate antibodies, aptamers could be employed. Aptamers are synthetic antibodies engineered through a selection process to have high binding affinity to their specific target.[35] They can be made from either peptides or nucleic acids. DNA aptamers are very stable compared to natural antibodies and could significantly improve the robustness of an antibody-based test for LRS. Antibody-based tests would be sickle specific, can provide quantitative results, and obviate the need for microscopes or protein gels.

Management

To be effective, improving methods for early detection mechanisms must be accompanied by increased access to management of the disease.

Comprehensive care

As mentioned above, it has been well established in higher-resource areas that NBS in conjunction with comprehensive care implemented quickly after diagnosis has been successful in improving outcomes and decreasing mortality due to SCD among children younger than five years of age.[1, 23] Comprehensive care includes health education, initiation of prophylactic penicillin, and administration of additional vaccinations and other preventive pharmacotherapeutic agents, as well as introduction of various screening tests. Along with NBS, adopting guidelines for follow-up care is important for decreasing early mortality due to SCD.

Health education

Even in the absence of other therapies, health education has been demonstrated to be effective in reducing SCD mortality in young children, preventing complications, and maintaining health through the life cycle.[1] This includes increasing knowledge to prevent dehydration, avoiding severe cold/heat, preventing infections, maintaining appropriate nutrition, recognizing and seeking early medical follow-up for symptoms, and implementing pain evaluation and management.[1]

Some individuals first learn that they are carriers when their child is born with SCD; therefore health education through the life cycle is important. Health education is also instrumental for

[i] RFA-HL-14-010, Developing a Point-of-Care Device for the Diagnosis of Sickle Cell Disease in Low Resource Settings SBIR (R43/ R44), identifies one possibly responsive application as "Low-cost, easy-to-use, portable lateral flow immunoassay (LFIA) POC blood test cassettes that provide results at the time of testing and use highly specific monoclonal antibodies (MAbs) to sickle hemoglobin (HbS), normal adult hemoglobin (HbA), and potentially HbF and HbC."

addressing misunderstandings and myths associated with SCD and decreasing stigma towards seeking care.

Vaccinations and prophylaxis (preventive pharmacotherapeutic agents)

As described earlier, most SCD patients develop functional asplenia which increases their susceptibility to life-threatening bacterial infections—many of which are preventable by vaccinations and regular prophylaxis.[1] Clinical guidelines for management of SCD include *Haemophilus influenzae* type b and pneumococcal vaccines, penicillin V prophylaxis (age two months to five years), and folic acid. In settings where malaria and intestinal parasitosis are common, antimalarials[12,15] and intestinal antiparasitics are also recommended[36,37] as SCD increases susceptibility to these health issues. Utilizing existing immunization infrastructure and strengthening health information systems that can track, remind, and recall SCD individuals for vaccinations and prophylaxis are feasible mechanisms for implementing these lifesaving measures.[38]

Other preventive measures

As mentioned earlier, pain due to VOEs can lead to very poor health-related quality of life as well as increased mortality, and it can be unpredictable.[8] There are few treatment options to prevent VOEs, and most are managed with traditional supportive care measures that have not changed over the decades. Novel management mechanisms are needed. One study reports potential promise utilizing microfluidic devices that could predict occurrence of painful VOEs.[39]

Following acute pain, acute coronary (chest) syndrome is the second most common cause of hospital admission for individuals with SCD. In addition, SCD is one of the most common causes of stroke in children. Renal damage is almost inevitable for SCD patients.[11] Preventive and management measures include transcranial Doppler (for pediatric patients) for stroke detection, complete pulmonary function test, annual ophthalmology exam, echocardiogram, complete blood count with differential and reticulocyte count, chemistries, ferritin level, hepatitis panel, and urinanalysis.[13] Technology innovations that enable such tests—especially for stroke detection and pulmonary function testing—to be more easily accessible to LRS would increase the ability of health programs to serve SCD individuals, preventing related morbidity and mortality. New developments in portable ultrasound, such as the Phillips Visiq,[40] could have a major impact if made more easily accessible.

Adult care

With increased survival rates into adulthood, adult health care for SCD patients is increasing in developed countries. The transition from pediatric to adult health providers has required provider education in order to meet that need. The most common causes of death in adults from SCD in high-resource countries include pulmonary hypertension, sudden death of unknown causes, renal failure, and infection.[41] Guidelines for management of adults with SCD are similar to those for children and include vaccination boosters and management of acute VOEs and complications. Severe pain associated with VOEs is the cause of nearly 90% of all hospital admissions among adult SCD patients in developed countries.[42] In addition, other health issues that may emerge for

adult patients include chronic lung disease; leg ulcers; or hepatic, endocrine, and even cardiac damage.[16]

Treatment

Currently, the only effective comprehensive treatment for SCD in high-resource countries includes hydroxyurea (also known as hydroxycarbamide) and supportive care for complications (analgesics, antibiotics, parenteral fluids, blood transfusion, and iron chelation).[6]

Hydroxyurea

Hydroxyurea is an anti-neoplastic that promotes the production of HgF (as do many cytotoxic drugs) and can be used to treat individuals with SCD.[11] It is considered the standard of care for patients with severe SCD and is the only drug approved by the US Food and Drug Administration for treatment of adults with SCD.[6] A number of clinical trials in developed countries have recently demonstrated the effectiveness of hydroxyurea in preventing complications of SCD, decreasing hospital admissions, and maintaining kidney function with few side effects in children as well as adults.[43,44] Trials are now starting in sub-Saharan Africa to demonstrate efficacy, dosing, and monitoring.[38]

This drug is an excellent treatment option for LRS since it can be administered orally, produced cheaply, and stored at room temperature. The current price of the drug is still high due to marketing practices, but it is anticipated that once efficacy is proven and hydroxyurea is recognized and adopted as the treatment standard for SCD, demand could drive price down.[38] Hence, a low-cost alternative to current hydroxyurea products presents an opportunity for improvement.

Administration of hydroxyurea in developed countries is usually administered with careful monitoring towards maintaining a therapeutic dosage. In LRS where this monitoring presents an additional burden and contributes to decreased access, trials are needed to demonstrate that the benefits of hydroxyurea administration outweigh the risks. In addition, a low-cost, simple test for monitoring hydroxyurea would be a promising and helpful tool in the treatment of SCD.[31] In high-resource settings, hydroxurea dosages are adjusted until the toxic threshold is reached, as evidenced by the onset of neutropenia. Current gold-standard methods for performing a complete blood count in the global north are not readily available in LRS; however, several groups (including PATH) have investigated charge-coupled device-based imaging cytometers as a low-cost alternative (including one that uses a cell phone as the detector).[45] So far, no clear leaders have emerged, but they are on the near horizon and may eventually address this shortfall.

There are a number of investigational agents at various stages of testing that have not yet proven to be of clinical benefit including drugs designed to increase hemoglobin F, short-chain fatty acids, medications to prevent sickle red blood cell dehydration, and anti-adhesion agents.[46]

Blood transfusion and iron chelation

Blood transfusions are used to manage acute and chronic complications for individuals with SCD in high-resource settings. Iron chelation addresses iron overload that results from chronic blood transfusions. These therapies are currently out of reach for most of the population in LRS and, therefore, present opportunities for technology innovations in this arena.

Bone marrow and stem cell transplant

Currently, the only cure for SCD is bone marrow and stem cell transplantation. In high-resource settings these are considered when serious complications have occurred, generally in children with cerebrovascular disease and transfusion dependence.[11] These are extremely costly and high-resource procedures and are consequently out of reach for most patients in low-resource areas.

Summary of opportunities for innovation

Across the continuum of care of SCD, there are many opportunities for technology innovation. Table 1 below shows opportunities for innovation across the continuum of care. Specifically, technologies appropriate for LRS would be very useful for preventive genetic testing of adults before conception as well as in pregnant women. Diagnostics for early detection, especially for newborns and young children, are key to identifying individuals with the disorder and initiating them into care, as well as differentiating from those who are carriers. While there are a number of diagnostics currently used in developed countries, technologies that can be applied in LRS—where most individuals with SCD live—are desperately needed; this means diagnostic tools that are low cost, do not need trained personnel, produce rapid and accurate results, and require minimal equipment and regents. For individuals diagnosed with SCD, tools for detecting and managing complications of SCD as well as treatment and potentially curative measures are largely inaccessible in LRS due to cost, complicated equipment, and the need for trained personnel to administer. Innovations in these tools could extend survival of individuals with SCD by improving management and prevention of SCD complications. Due to the extent of the disease, the impact any one of these technology innovations could make on morbidity and mortality—especially in the geographic regions most affected—is significant.

Table 1: Sickle cell disease opportunities for innovation across the continuum of care.

	Standard of care	Opportunities for innovation
Prevention	• Health education and counseling • Carrier screening • Genetic testing for adults • Prenatal screening	• Novel genetic testing and screening mechanisms for preconception and pregnant couples
Early detection	• Hemoglobin analysis techniques: - Hemoglobin electrophoresis - Isoelectric focusing - High-performance liquid chromatography • Microscopy • Point of care	• Better diagnostics for neonates and young children: - Paper-based solubility tests - P (sickle)-based test - Antibody-based tests, esp. lateral flow strip or other microfluidic devices - Aptamer-based tests
Management	• Health education • Preventive agents: - Vaccines, - Penicillin V - Folic acid, - Antimalarials - Intestinal antiparasitics • Tests for complications: - Transcranial Doppler - Pulmonary function test - Annual eye exam - ECHO - Complete blood count - Blood chemistries - Ferritin level - Hepatitis panel - Urinanalysis	• Microfluidic devices (for predicting occurrence of vaso-occlusive episodes) • Technology innovations (especially for stroke detection and pulmonary function testing), e.g., portable ultrasound (Philips Visiq)
Treatment	• Disease modifying agents and monitoring mechanisms - Hydroxyurea • Supportive care - Analgesics - Antibiotics - Parenteral fluids - Blood transfusion - Iron chelation	• Nutrition • Low-cost alternative to current hydroxyurea products • Low-cost simple imaging cytomter for monitoring hydroxyurea titration via neutropenia • Investigational agents: - Drugs to increase fetal hemoglobin - Short chain fatty acids - Medications to prevent sickle red blood cell dehydration - Anti-adhesion agents • Blood transfusion and iron chelation alternatives
Cure	• Curative measures - Bone marrow and stem cell transplant	• Low-cost alternatives to transplant procedures

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