COMMENTARY

A diagnostic role for dense cells in sickle cell disease

Natasha M. Archer[1]

Dana–Farber/Boston Children's Cancer and Blood Disorders Center, Boston, MA 02215

Neonatal screening for sickle cell disease (SCD), when linked to early diagnostic testing, parental education, and comprehensive care, markedly reduces morbidity and mortality from the disease in infancy and early childhood (1, 2). Largely unaddressed, the inability to diagnose SCD rapidly, reliably, and at low cost continues to be a significant contributor to high morbidity and mortality in many resource-limited settings. Until recently, few practical and reliable point-of-care methods for the diagnosis of SCD existed. The recent call by major organizations, such as the National Heart, Lung, and Blood Institute and the World Health Organization for simple, rapid and low-cost diagnostic techniques for SCD, has led to more research dedicated to solving this global problem. In 2013, Milligan et al. proposed the use of hemolysis in deoxygenated isosmotic sucrose solution as a method to diagnose SCD (3). In that same year, Yang et al. described a paper-based solubility test able to differentiate between SCD, sickle cell trait, and nonhemoglobin S-containing blood (4). In PNAS, Kumar et al. present an innovative approach to SCD point-of-care testing using density gradients created by aqueous multiphase systems (AMPS) (5).

Traditionally, diagnosis has relied upon the detection of hemoglobin S, but using factors specific to the red cells in patients with SCD may be more practical given the varying levels of hemoglobin S throughout the lifetime of a SCD patient. Kumar et al. (5) take the notoriously dreaded dense SCD cells that characterize the disease and transform them into a biomarker. The authors also repurpose AMPS to create density gradients that can detect these dense cells. This novel system allows the operator to visually distinguish between SCD and nondisease (genotype Hb AA and Hb AS) in less than 12 min with greater than 90% sensitivity (Fig. 1). The proposed new methodology has potential utility in resource-limited settings as a result of its rapidity, portability, and electricity independence, as well as in high-income countries where it can potentially facilitate more effective management of severely affected individuals. However, limitations exist, including the impracticality in newborns and potential for decreased sensitivity in real-world settings, both of which will need to be addressed before it replaces more traditional forms of diagnosis, such as isoelectric focusing.

Most helpful in meeting the needs of SCD programs in resource-limited settings is the rapidity of the proposed test. Rapid SCD screening is not needed in high-income countries, like the United States, where preventative care is a well-established health literacy concept. However, in many resource-limited settings the idea of actively seeking blood test results when a baby or child is not apparently ill is a foreign and ill-advised concept. Previous groups have set up well-structured, SCD management programs in resource-limited settings but have struggled with low return rates (6, 7). A rapid test allows results to be communicated and care to be initiated during the same visit in which testing has occurred. In addition, the test's portability and electricity independence present the option for community health workers to use this test in the field. Going home-to-home and testing babies and young children as opposed to traditional hospital-based programs will greatly impact the number of children screened in resource-limited settings where the majority of births happen outside of the hospital.

However, most newborns have a fetal hemoglobin percentage of 50–80%. In the presence of such a high hemoglobin F, dense SCD cells are unlikely to exist. Although the system

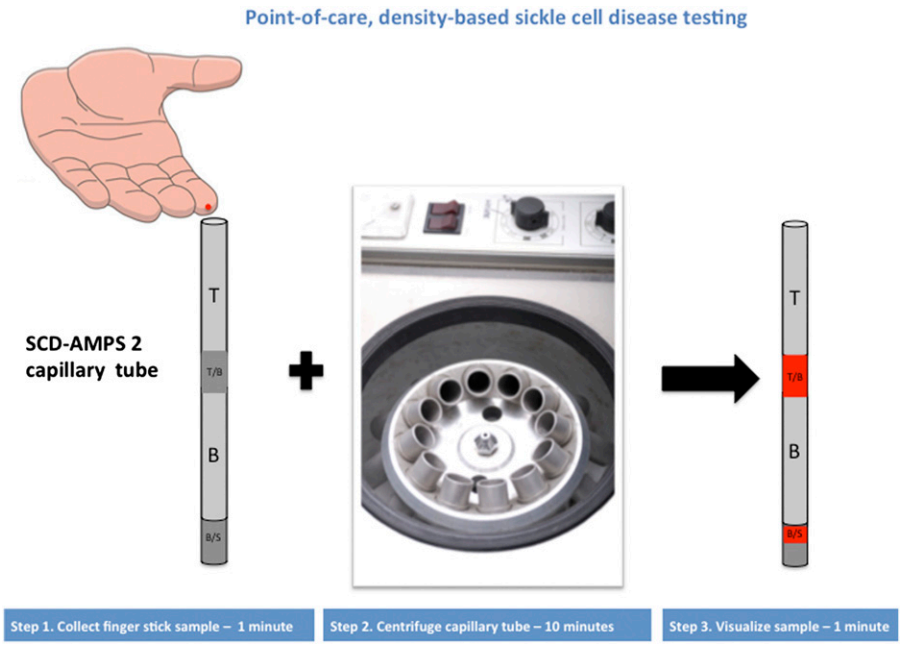

Fig. 1. Example of the SCD-AMPS 2 rapid test. A capillary blood sample from a finger stick is loaded into a capillary blood tube preloaded with SCD AMPS that has been sealed at one end. The capillary tube is centrifuged for 10 min. The red blood cells settle into their respective density phases (T, top; B, bottom) or interfaces (T/B, top/bottom; B/S, bottom/seal). In SCD^{+} blood (shown), erythrocytes settle in the T/B interface and the B/S interface. In non-SCD blood (not shown), the erythrocytes only settle in the T/B interface. SCD-AMPS 3 is not shown.

Author contributions: N.M.A. wrote the paper.

The author declares no conflict of interest.

See companion article on page 14864.

[1]Email: Natasha_Archer@dfci.harvard.edu.

proposed by Kumar et al. (5) cannot be used for neonatal diagnosis, this is potentially a reliable test in infants greater than 6 mo of age and adults. Newborn screening is clearly the ideal, but the greatest mortality risk to infants with SCD occurs in children 6–12 mo of age (8). Detection just before 6 mo of age, when the fetal hemoglobin percentage is <10% and dense cells are expected to be present, will allow for the allocation of life-saving interventions in time to prevent significant morbidity and mortality.

Although controversial, genetic counseling can lead to a large-scale reduction in births of affected children (9). The reality of SCD is that it can be prevented. Couples at risk for having affected children can be identified using this test and counseled accordingly. If couples do decide to have a child, counseling may at the very least encourage more families to seek results, even if newborn screening occurs via less-rapid testing, such as isoelectric focusing, hemoglobin electrophoresis, or high-performance liquid chromatography. The test can also be useful before surgical procedures and childbirth, potentially altering transfusion protocols and management in the operating room of individuals with SCD.

This method also has the potential to impact the care of patients with SCD living in high-income countries. Density has been associated with certain specific sickle cell syndromes (10). Stem cell transplant (SCT) is the only available cure for SCD. With an inherent mortality risk of up to 10%, SCT is most often only offered to individuals with severe disease (i.e., a history of multiple acute chest syndromes, debilitating vaso-occlusive crises, or stroke). At the point of SCT, many SCD patients have evidence of end organ damage that can further increase the mortality risk associated with SCT. This test may be able to identify potential SCT candidates prior to the development of morbid SCD complications. Hydroxyurea, the most effective treatment at present for SCD, can also be initiated early in the course of disease, thus preventing significant morbidity and mortality.

The system has its limitations. At 90% sensitivity in a controlled setting, the system proposed already has a moderately high rate of false-negatives that will undoubtedly increase once used in the field. Concurrent medical conditions, such as iron-deficiency anemia, can also decrease test sensitivity as it decreases the amount of dense cells present. This is a major limitation. Iron deficiency anemia is widely prevalent worldwide, especially in resource-limited countries where SCD is most common (11). The more false-negatives that exist, the more young children go undiagnosed.

Kumar et al. take the notoriously dreaded dense SCD cells that characterize the disease and transform them into a biomarker.

Kumar et al. (5) tout low cost and simplicity as major attributes of the system. The cost of $0.50, although cheaper than conventional methods, is still cost-prohibitive in many countries where the average daily income is less than $1 per day. The test requires a trained professional to perform and interpret the results. In order for a 5-μL fingerstick capillary blood sample to be directly uploaded into a pretreated capillary tube, one must know how to perform a finger stick and how to manipulate a capillary blood tube. Phlebotomist and laboratory personnel in resource-limited settings can definitely handle this task and can be trained to feel confident making the visual call of disease or no disease. However, this currently falls out of the range of tasks for the average community health worker operating in the field. If that indeed is the goal, significant operational and analytical training will be required.

SCD is a major global health problem, particularly in countries with high malaria endemicity (12). Life-saving interventions cannot be universally implemented until we are able to systematically diagnose individuals living in highly prevalent areas. Kumar et al. (5) propose a long-overdue potential solution to the problem of SCD diagnosis. Further research into improving sensitivity in real-world settings and detecting low levels of dense cells is needed to enable use in newborns. However, this is definitely one big step toward improved care for patients worldwide with SCD, even if only applicable to young children and adults.

1 Vichinsky E, Hurst D, Earles A, Kleman K, Lubin B (1988) Newborn screening for sickle cell disease: Effect on mortality. *Pediatrics* 81(6): 749–755.

2 Quinn CT, Rogers ZR, McCavit TL, Buchanan GR (2010) Improved survival of children and adolescents with sickle cell disease. *Blood* 115(17):3447–3452.

3 Milligan C, et al. (2013) A non-electrolyte haemolysis assay for diagnosis and prognosis of sickle cell disease. *J Physiol* 591(Pt 6): 1463–1474.

4 Yang X, et al. (2013) A simple, rapid, low-cost diagnostic test for sickle cell disease. *Lab Chip* 13(8):1464–1467.

5 Kumar AA, et al. (2014) Density-based separation in multiphase systems provides a simple method to identify sickle cell disease. *Proc Natl Acad Sci USA* 111:14864–14869.

6 Tshilolo L, et al. (2009) Neonatal screening for sickle cell anaemia in the Democratic Republic of the Congo: Experience from a pioneer project on 31 204 newborns. *J Clin Pathol* 62(1):35–38.

7 McGann PT, et al. (2013) A prospective newborn screening and treatment program for sickle cell anemia in Luanda, Angola. *Am J Hematol* 88(12):984–989.

8 Serjeant GR; GR (2013) The natural history of sickle cell disease. *Cold Spring Harb Perspect Med* 3(10):a011783.

9 Memish ZA, Saeedi MY (2011) Six-year outcome of the national premarital screening and genetic counseling program for sickle cell disease and β-thalassemia in Saudi Arabia. *Ann Saudi Med* 31(3):229–235.

10 Bartolucci P, et al. (2012) Erythrocyte density in sickle cell syndromes is associated with specific clinical manifestations and hemolysis. *Blood* 120(15):3136–3141.

11 Kassebaum NJ, et al. (2014) A systematic analysis of global anemia burden from 1990 to 2010. *Blood* 123(5):615–624.

12 Piel FB, et al. (2013) Global epidemiology of sickle haemoglobin in neonates: A contemporary geostatistical model-based map and population estimates. *Lancet* 381(9861): 142–151.