The Oxygenscan: A Rapid and Reproducible Test to Determine Patient–Specific, Clinically Relevant Biomarkers of Disease Severity in Sickle Cell Anemia

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Abstract

Background: In sickle cell anemia (SCA), hemoglobin S (HbS) polymerizes upon deoxygenation, resulting in sickling of red blood cells (RBCs). These deoxygenated RBCs have strongly reduced deformability, which contributes to the etiology of vaso-occlusive crises and chronic hemolytic anemia. There are no widely available clinical laboratory tests to directly monitor effects of disease modifying therapies (i.e. hydroxyurea) on RBC deformability. RBC deformability can be measured using a Laser Optical Rotational Red Cell Analyzer (Lorrca) ektacytometer (RR Mechatronics, the Netherlands), which measures RBC deformability over a range of osmolalities. Recently, a new module was added which consists of a method to measure RBC deformability, expressed as Elongation Index (EI), during controlled deoxygenation. This test, termed oxygenscan, has 3 key read out parameters: 1) EImax, which represents RBC deformability at normoxia; 2) EImin represents deformability upon deoxygenation; and 3) the point of sickling (PoS), the point at which a >5% decrease in EI is observed during deoxygenation, reflecting the patient-specific pO2 at which sickling begins (Figure 1). In this study, we correlated laboratory parameters associated with SCA disease severity with oxygenscan parameters to establish the clinical utility of this test.

Methods: The discovery cohort consisted of 15 SCA patients (median age 22.0 years, 33.3% on hydroxyurea (HU)) enrolled at University Medical Center Utrecht (UMCU). The validation cohort consisted of 21 patients with SCA (median age 12.5 years, 76.2% on HU) from Texas Children's Hematology Center (TCHC). Oxygenscans were carried out in duplicates at both sites. Percentage dense RBC (%DRBC) were measured using an ADVIA hematology analyzer (Siemens) at TCHC only. In this study, we used Pearson's correlation to test for linear correlations between oxygenscan parameters EImax, EImin and PoS and clinically relevant laboratory parameters: total hemoglobin (Hb), absolute reticulocyte count (ARC), %HbS and %HbF, and %DRBC.
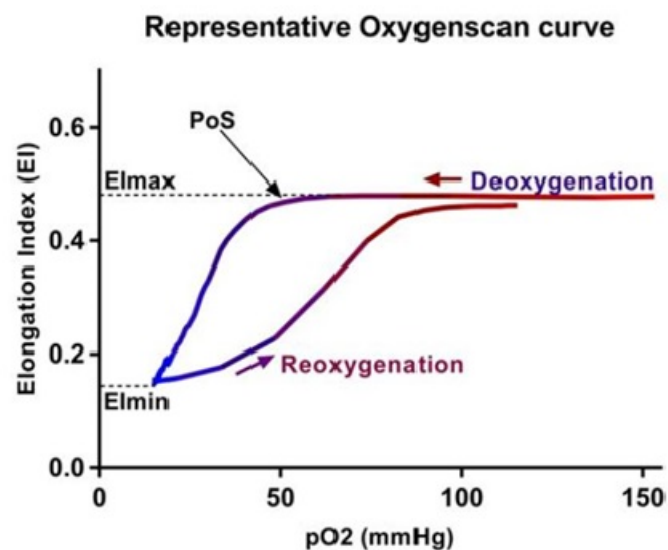
Figure 1. An oxygenscan starts with deoxygenation followed by reoxygenation. EImax reflects overall deformability of the total RBC population. EImin represents minimal deformability due to change in shape and orientation of RBCs upon deoxygenation. Point of Sickling (PoS) embodies the oxygen tension when the first RBCs start to sickle.

Results: In both cohorts PoS significantly positively correlated with ARC (Figure 2A-B). In the UMCU cohort, total Hb levels also significantly positively correlated with EImax (Figure 2C), which was validated in the TCHC cohort (Figure 2D). HbF positively correlated with the EImin in both cohorts (Figure 2E-F). EImin also significantly negatively correlated with HbS (r=-0.828 p=<0.001 in the UMCU cohort, r=-0.936, p=<0.001 in the TCHC cohort data not shown). EImax showed a strong negative correlation with the %DRBC (Figure 2G) in the TCHC cohort. Individual test results were highly reproducible at both sites, with a median coefficient of variability of all tested parameters below 3%.

Conclusion: The oxygenscan is a semi-automated, inexpensive, highly reproducible, and rapid test to fully characterize patient-specific RBC deformability under a range of oxygen concentrations. Key oxygenscan measurements- PoS, EImin, and EImax- correlated with known measures of SCA disease severity, namely ARC, HbF, HbS, total Hb and %DRBC. Patients with higher reticulocyte counts showed a clinically unfavorable increase of oxygen concentration at which RBCs start to sickle (termed PoS), than patients with lower ARC. Patients with higher HbF had more deformable RBCs even at the lowest oxygen concentrations, or EImin, while patients with higher HbS had lower EImin (low values indicate poor deformability under deoxygenated conditions). Patients with high %DRBC had lower EImax, indicating poor RBC deformability at normoxic conditions. Conversely, patients with high total Hb had high EImax.

The very strong correlations of key oxygenscan measurements with different measures of SCA disease severity suggest that these parameters could be exploited as useful biomarkers of clinical severity and in the follow-up and treatment of SCA patients and warrant further investigation.
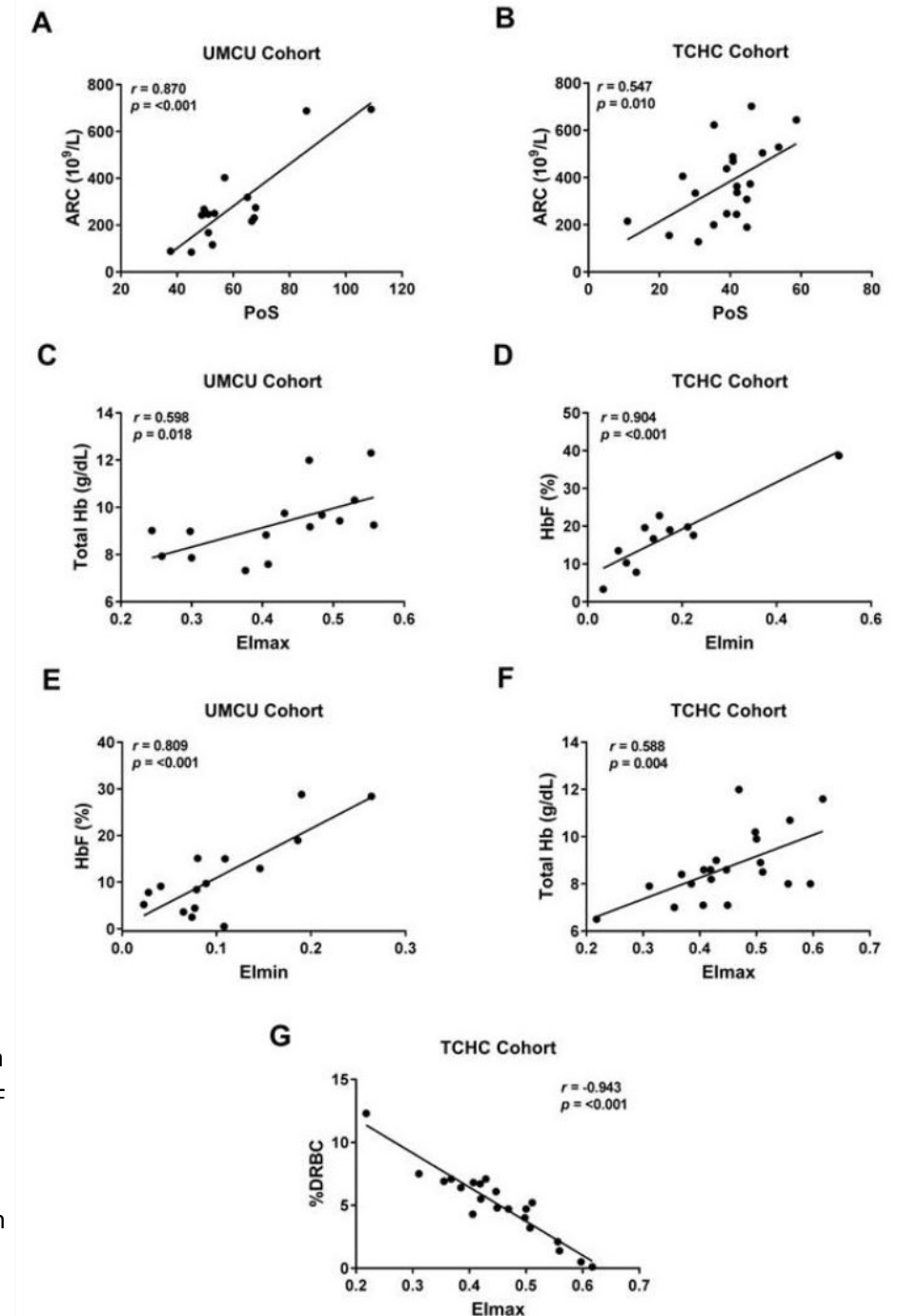
Figure 2. Linear correlations of PoS with ARC in UMCU cohort (A) and TCHC cohort (B), of EImax with total Hb (C and D), of EImin with HbF (E and F) and EImax with %DRBC (G).