ELSEVIER

Blood 142 (2023) 2497–2498

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

Roxyscan: A Novel Method to Assess Red Blood Cell Resilience to Oxidative Stress in Sickle Cell Disease

Carolina A. Hernandez[1], *Marissa J.M. Traets*[1], *Myrthe J. van Dijk*[2,1], *Wouter W. van Solinge*[1], *Frans A. Kuypers, PhD*[3], *Minke A.E. Rab*[4,1], *Eduard J. Van Beers, MDPhD*[5], *Richard van Wijk*[1]

[1] Central Diagnostic Laboratory - Research, University Medical Center Utrecht, Utrecht, Netherlands
[2] Center for Benign Hematology, Thrombosis and Hemostasis - Van Creveldkliniek, University Medical Center Utrecht, Utrecht, Netherlands
[3] Children's Hospital Oakland - Research Inst., Oakland, CA
[4] Department of Hematology, Erasmus MC, Rotterdam, Netherlands
[5] Center for Benign Hematology, Thrombosis and Hemostasis - Van Creveldkliniek, University Medical Centre Utrecht, Utrecht, Netherlands

Background: Red blood cells (RBCs) are biconcave-shaped cells with viscoelastic membranes that are optimally adapted for oxygen delivery and gas exchange during their normal lifespan of 120 days. Reactive oxygen species (ROS) continuously challenge the viability of RBCs. Despite an arsenal of compounds to protect the RBC, including Glutathione (GSH), Vitamin E, superoxide dismutase (SOD) and Catalase, damage can easily be inflicted that will disrupt cellular deformability, structure, and membrane properties. The susceptibility to oxidative stress in individuals with Sickle Cell Disease (SCD) is believed to be linked to multiple factors. These include the release of heme and iron during hemolysis, inflammation processes, and a reduction in antioxidant defenses.
Methods:Susceptibility of RBCs to oxidative stress was assessed with the RoxyScan. This novel application of the Lorrca MaxSis (RR Mechatronics) measures rheological behavior of RBCs in response to cumene hydroperoxide (CuHP, 100 μM) exposure during continuous shear (30 Pa). For this we used the Lorrca's peristaltic pump and suction needle, directing the blood and CuHP to the instrument's cup and mixing them for 30 seconds (s) before starting the run. Deformability of RBCs (EI, elongation index) was measured over time. PoX is defined as the time where a 10% decrease in EI is reached. EI start: EI t=0. EI final: EI t=1600s, ΔEI (EIstart-EIfinal). RoxyScan measurements were performed on blood samples from 16 adult SCD patients (HbSS, HbS/β0 or HbS/β+) and 19 healthy controls (HC). Outcome measurement PoX (calculated from curve fitted data) was correlated to laboratory parameters from complete blood count (Cell Dyn Sapphire, Abbott), RBC sickling tendency (Point of Sickling, PoS, as assessed by oxygen gradient ektacytometry on the Lorrca MaxSis), and levels of HbF/HbS (Tosoh G8).
Results:RBCs from untreated HC show a stable EI over time (Fig 1A). Upon exposure to CuHP a similar decrease in EI is seen in 19 HC (Fig 1A), mean decrease 0.055, range (0.017 to 0.10). The mean and median calculated PoX in HC is 1723s and 1702s respectively. In contrast, SCD RBC showed an earlier and more pronounced decrease in EI in response to CuHP exposure (representative example, Fig 1A). Comparing the PoX between HC and SCD revealed significant differences: mean PoX HC: 1723s (SD 329s), SCD: 725s (SD 472s), ***p<0.0001, indicating that SCD RBCs are more susceptible to oxidative stress. Correlating PoX to routine laboratory parameters identified a correlation with Hb levels (***p=0.002), inverse correlation with reticulocyte count (***p=0.0014) and hemoglobin distribution width (HDW, ***p<0.001) (Fig 1B). Similarly strong inverse correlations were found for EI start and EI end (***p<0.001) (Fig 1B). PoX also showed an inverse correlation with red cell distribution width (RDW, ns p=0.07) and mean corpuscular volume (MCV, ns p=0.77). Interestingly, PoX showed a significant inverse correlation with PoS (***p=0.004), indicating that SCD RBCs which are more susceptible to oxidative stress tend to sickle at a higher oxygen tension. PoX showed no significant correlations with HbS/HbF levels (Fig 1B).
Conclusion: In this study we demonstrate the applicability of the RoxyScan as a novel method to assess RBC resilience to oxidative stress. When applied to SCD, RBCs show an earlier and more pronounced response to oxidative stress than RBCs from HC. The inverse correlation of PoX, the major outcome parameter of this technique, with reticulocyte count could indicate a generally increased susceptibility to oxidative stress due to a higher level of hemolysis. The correlation with Hb levels affirms this. PoX showed other strong and significant correlations with routine laboratory parameters, such as HDW, implying that the greater variability in hemoglobin content is associated with higher susceptibility to oxidative stress. On a functional level,

it is interesting to find strong correlations of PoX with PoS, showing a direct link between RBC sickling tendency and ability to withstand oxidative stress. On the other hand, HbS and HbF levels did not correlate significantly to PoX. Overall, our study highlights the potential use of the RoxyScan and its associated biomarkers in the analysis of the RBC's response to oxidative stress. Further research is in progress to investigate the clinical applicability of this new technique.

Disclosures van Dijk: *Axcella Health Inc.:* Research Funding; *Agios Pharmaceuticals Inc.:* Research Funding. **Kuypers:** *GBT/Pfizer:* Research Funding; *Forma/NovoNordisk:* Research Funding. **Rab:** *Axcella Therapeutics, Inc.:* Research Funding; *RR Mechatronics:* Research Funding; *Pfizer, Inc.:* Research Funding; *Agios Pharmaceuticals, Inc.:* Consultancy, Research Funding. **Van Beers:** *Agios Pharmaceuticals, Inc. Inc.:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Pfizer, Inc.:* Research Funding; *Novartis AG:* Research Funding; *RR Mechatronics:* Research Funding. **van Wijk:** *Axcella Therapeutics, Inc.:* Research Funding; *RR Mechatronics:* Research Funding; *Pfizer, Inc.:* Consultancy, Research Funding; *Agios Pharmaceuticals, Inc.:* Consultancy, Research Funding.

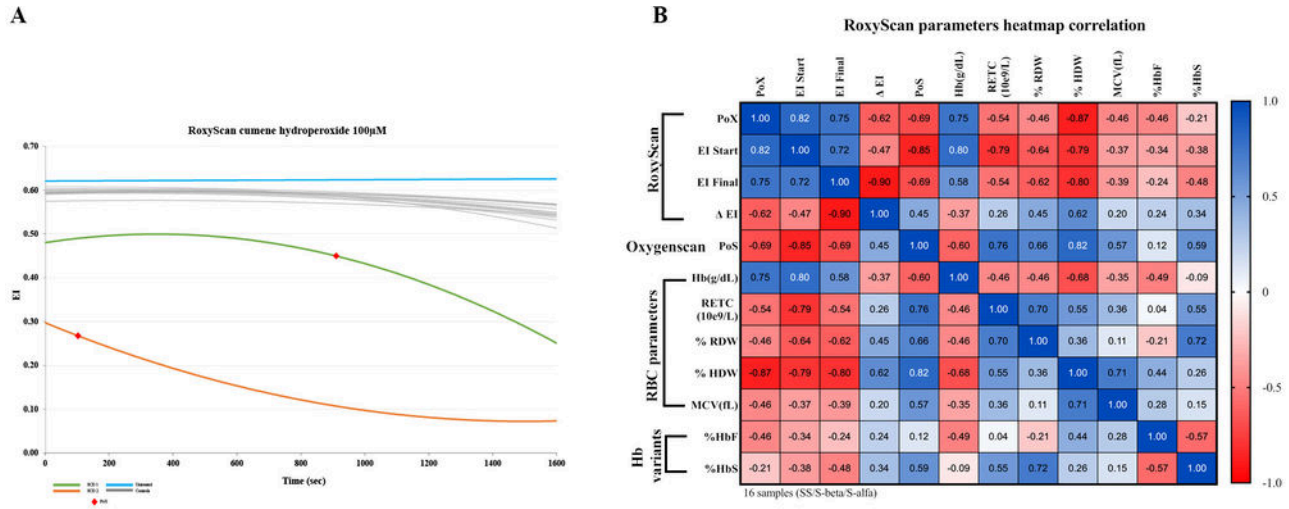

Figure 1. RoxyScan characterization of deformability and PoX correlations and groups evaluation. Results for 1600 seconds assay in three conditions: untreated healthy control (HC) blood as blank, and cumene hydroperoxide titration in HC and Sickle Cell disease (SCD) blood **A.** RoxyScan EI changes in the increase of oxidative stress (PoX in red)**.** Results for 1600 seconds assay in cumene hydroperoxide titration in Sickle Cell disease (SCD) blood correlations. **B.** correlations heatmap between the RoxyScan, oxygenscan, extended RBC parameters and Hb variants.

Figure 1

https://doi.org/10.1182/blood-2023-188676