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Comment on: Oxygen gradient ektacytometry does not predict pain in children with sickle cell anaemia

We read with great interest the recently published report of Nardo-Marino *et al.*[1] The authors report results of a study designed to: (1) investigate the relationship between oxygen gradient ektacytometry-derived biomarkers and two established markers of disease severity in children with sickle cell anaemia (SCA), (2) examine the impact of hydroxycarbamide (hydroxyurea [HU]) on red blood cell (RBC) deformability measured with oxygen gradient ektacytometry and (3) investigate the ability of oxygen gradient ektacytometry-derived parameters to predict pain as a clinical outcome in SCA. The authors found that all oxygen gradient ektacytometry-derived parameters correlated with known modifiers of disease severity, and that specifically the point of sickling (PoS) correlated with transcranial Doppler velocities. On the other hand, they did not find that oxygen gradient ektacytometry parameters independently predicted pain events or response to HU treatment. Therefore, the authors concluded that oxygen gradient ektacytometry could not predict pain events in SCA and questioned its use in clinical practice.

Oxygen gradient ektacytometry is a technique that is sensitive to a number of factors and requires a certain degree of expertise to be carried our properly.[2,3] We also are aware that the use of oxygen gradient ektacytometry in clinical practice has not been established. However, we are concerned by the overall conclusions drawn by the authors, which we strongly believe are premature because of limitations in the study design and interpretation of the results.

Firstly, the authors found no association of oxygen gradient ektacytometry-derived parameters with HU treatment. No other laboratory parameters were analysed to investigate if there was a (significant) difference between the HU and non-HU treated cohorts. More importantly, the authors did not investigate if pain rate was significantly lower in the HU-treated cohort. As already pointed out by the authors, to fully establish if oxygen gradient ektacytometry-derived parameters can serve as biomarker for efficacy of HU therapy, a longitudinal study design is needed. Recently, such a study has been carried out. This study, in 15 individuals with SCD, found a marked effect of HU on oxygenscan parameters, with a mean decrease of PoS of 13.7 mmHg ($p < 0.0001$) after 6 months of HU therapy.[4]

Secondly, the authors observed no significant association of oxygen gradient ektacytometry-derived parameters with pain episodes. In the statistical analysis, the authors correct for age, but also for levels of haemoglobin (Hb), percentage fetal Hb (%HbF) and HU treatment. Oxygen gradient ektacytometry-derived parameters are the result of numerous RBC characteristics including %HbF, percentage sickle Hb (%HbS), mean corpuscular volume, mean corpuscular Hb concentration, dense RBCs and irreversibly sickled cells (ISCs). Levels of HbF are integral to rates of sickling and therefore cannot be corrected for in a regression analysis. Moreover, functional RBC analysis such as oxygen gradient ektacytometry provides more information on how %HbF relates to other biomarkers and could also explain why some individuals with high HbF levels still experience pain crisis. This pain crisis could be explained by the distribution of HbF or other RBC characteristics that can be captured by oxygen gradient ektacytometry and other functional biomarkers such as blood viscosity and that is also dependent on multiple factors including RBC characteristics.

Pain crises were evaluated in the past 2 years, and for self-reported pain presented as daily, weekly, monthly, and yearly during the past 12 months. However, their study included children from the age of 7 months. This will confound results because the SCA phenotype generally manifests at >6 months after birth, with concomitant oxygenscan findings, when no 2-year history of pain is present. Furthermore, as the authors also discussed, self-reported pain is not commonly used as outcome as it is very subjective and is challenging to assess in very young children.

Furthermore, the authors conclude that based on their findings, oxygen gradient ektacytometry does not provide any additional information in predicting the clinical course in children with SCA, beyond measuring known markers of disease severity such as %HbF. However, they do not demonstrate that %HbF has predictive value in their data set. It is possible that the problem lies in a small and noisy dataset, and not in oxygen gradient ektacytometry. Similarly, did the authors find significant and relevant odds ratios of other markers of disease severity, such as levels of Hb and co-inheritance of α-thalassaemia in relation to pain?

It would be helpful to provide additional data of other laboratory parameters with regard to the eight patients whose RBCs have a high elongation index (EI) during a hypoxic state (EI_{min} >0.4) and low EI under normal oxygen tensions (EI_{max} <0.6). These characteristics could be considered favourable, as suggested by the authors. However, six children

reported a pain crisis in the past 2 years, one of whom was admitted for pain four times in past 12 months. Before conclusions can be drawn on the usefulness of oxygen gradient ektacytometry, one would need to know the levels of HbF, HbS, HbA and total Hb, as well as α-thalassaemia status. The latter is thought to be a favourable genetic modifier of SCA; however, its co-inheritance is associated with an increased risk of pain crises,[5] similar as high Hb levels.[6]

Finally, another possible confounder in this study is the storage time of >24 h before the samples were measured, as the authors themselves also discuss.[7,8] During transportation and long storage time the most severely affected RBCs are more prone to haemolysis and there is possibly an accumulation of ISCs. This lowers the discriminative power of the oxygenscan, as the RBCs that lyse are the same RBCs that sickle at high oxygen tensions and have a large influence on PoS. The accumulation of ISCs would surely impact on oxygen gradient ektacytometry parameters.

The authors conclude that there is no place for oxygen gradient ektacytometry as biomarker for prediction of pain and that the technique cannot replace patient-reported outcomes in clinical and clinical-trial settings. It is our opinion that the study was not designed appropriately to be able to predict a complication because it is a retrospective cohort study. Such a study can merely find associations between laboratory parameters and occurrence of clinical complications in the past, if large enough and designed appropriately. In that respect the title of the report is inaccurate.

With regard to the highly complex pathophysiology of SCA it would be unreasonable to assume that a single biomarker would be able to predict all different complications of this disease. Known markers of disease severity reflect different characteristics of sickle cell disease and the combination of those markers are applied in clinical trials even though those markers relate to different complications. For instance, Hb, a marker of anaemia, is not associated with pain crises, but is widely applied in clinical care. Similarly, United States Food and Drug Administration (FDA) approval of voxelotor has been granted based on the increase in Hb levels.[9] Oxygen gradient ektacytometry is a laboratory test that can characterise and quantify sickling, which reflects the pathognomal feature of SCA. As such, oxygen gradient ektacytometry-derived parameters can complement known markers of SCA. Currently ongoing and future (prospective, longitudinal) studies are warranted to firmly establish if oxygen gradient ektacytometry-derived parameters are predictive of pain and other SCA-related complications and of additional value beyond known markers of disease severity in SCA.

KEYWORDS
hydroxycarbamide, oxygen gradient ektacytometry, red blood cell deformability, sickle cell anaemia, α-thalassaemia

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REFERENCES

1. Nardo-Marino A, Petersen J, Brewin JN, Birgens H, Williams TN, Kurtzhals JA, et al. Oxygen gradient ektacytometry does not predict pain in children with sickle cell anaemia. Br J Haematol. 2021 (Online ahead of print). https://doi.org/10.1111/bjh.17975
2. Rab MA, van Oirschot BA, Bos J, Kanne CK, Sheehan VA, van Beers EJ, et al. Characterization of sickling during controlled automated deoxygenation with oxygen gradient ektacytometry. J Vis Exp. 2019;153:1–10.
3. Rab MA, van Oirschot BA, Bos J, Merkx TH, van Wesel AC, Abdulmalik O, et al. Rapid and reproducible characterization of sickling during automated deoxygenation in sickle cell disease patients. Am J Hematol. 2019;94:575–84.
4. Rab MA, Kanne CK, Bos J, van Oirschot BA, Boisson C, Houwing ME, et al. Oxygen gradient ektacytometry derived-biomarkers are associated with vaso-occlusive crises and correlate with treatment response in sickle cell disease. Am J Hematol. 2021;96:E29–32.
5. Piel FB, Steinberg MH, Rees DC. Sickle cell disease. N Engl J Med. 2017;376:1561–73.
6. Kalpatthi R, Novelli EM. Measuring success: utility of biomarkers in sickle cell disease clinical trials and care. Hematology. 2018;2018:482–92.
7. Rab MA, Kanne CK, Bos J, Boisson C, van Oirschot BA, Nader E, et al. Methodological aspects of the oxygenscan in sickle cell disease: a need for standardization. Am J Hematol. 2020;95:E5–8.
8. Boisson C, Rab MA, Nader E, Renoux C, van Oirschot BA, Joly P, et al. Methodological aspects of oxygen gradient ektacytometry in sickle cell disease: effects of sample storage on outcome parameters in distinct patient subgroups. Clin Hemorheol Microcirc. 2021;77:391–4.
9. Vichinsky E, Hoppe CC, Ataga KI, Ware RE, Nduba V, El-Beshlawy A, et al. A phase 3 randomized trial of voxelotor in sickle cell disease. N Engl J Med. 2019;381:509–19.