Use of the oxygen gradient ektacytometry in the dose titration of hydroxyurea therapy in children with sickle cell disease

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LETTER TO THE EDITOR

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Dear Editors,

International guidelines recommend that hydroxyurea should be offered to all children with HbSS or HbSβ^0 sickle cell disease at 9 months of age regardless of clinical course. However, there are substantial gaps in our knowledge with regard to optimal dosing, dose modifications and verification of adherence to treatment.

Sickle cell disease is an autosomal, recessive hemoglobinopathy characterized by ongoing hemolytic anemia, episodes of vaso-occlusive crises and progressive organ failure. Worldwide, approximately 312.000 neonates are born each year with this disease.[1] Sickle cell disease is caused by a single nucleotide substitution in codon 6 of the β globin gene (HBB:c.20A > T, p.Glu7Vall). This mutation leads to the formation of abnormal sickle hemoglobin (HbS). When deoxygenated, HbS containing red blood cells (RBCs) develop a sickle or crescent shape. Such abnormally shaped RBCs interact with leukocytes and the vascular endothelium causing occlusion and vasculopathy, subsequently leading to a broad range of acute and chronic complications.

Long-term daily oral hydroxyurea treatment has consistently been shown to be clinically effective and to positively affect laboratory values in pediatric patients with sickle cell disease. Patients experience less frequent vaso-occlusive pain- and acute chest episodes, require fewer hospitalizations and blood transfusions and show increased levels of total hemoglobin (Hb) and fetal hemoglobin (HbF) and decreased levels of lactate dehydrogenase and bilirubin.[2] Laboratory values, such as Hb, HbF levels and mean corpuscular volume (MCV) are frequently used in clinical practice to assess treatment efficacy. However, none of these routine tests capture the overall effect hydroxyurea has on RBCs.

We present a case report comprising of monozygous twin girls, 6 years old, treated at the Sickle Cell Disease Comprehensive Care outpatients' clinic of the Erasmus MC-Sophia Children's Hospital for their biannual follow-up visit. They were diagnosed with homozygous sickle cell disease (HbSS) soon after birth by neonatal screening. Except for a transient aplastic crisis associated with parvovirus B19 infection when they were 4 years old, they had an unremarkable medical history without frequent pain crises or organ failure, including normal transcranial Doppler values. Due to the modification of the international guidelines in 2014[3] and subsequent national guideline adaptation in The Netherlands regarding initiation of hydroxyurea therapy in young children with sickle cell disease at 9 months of age, the twins started with hydroxyurea at a dosage of 22.4 and 19.8 mg/kg respectively, when they were 6 years of age. Besides standard routine laboratory parameters we measured RBC sickling behavior with oxygen gradient ektacytometry. Parents gave informed consent to include oxygen gradient ektacytometry measurements during regular follow-up. Oxygen gradient ektacytometry measures deformability of RBCs over a range of oxygen concentrations using a Laser Optical Rotational Red Cell Analyser (Lorrca) ektacytometer (RR Mechatronics, Zwaag, the Netherlands).[4] Oxygen gradient ektacytometry produces three key parameters: (1) EI_{max}, RBC deformability at the start of the measurement at normal oxygenation; (2) EI_{min}, deformability upon deoxygenation; (3) Point of Sickling (PoS): oxygen tension at which 5% decrease in EI is observed during deoxygenation, reflecting the patient-specific pO_2 at which cells become more rigid and sickling begins. The fourth and novel parameter is called Slope, indicating the velocity of RBC sickling during deoxygenation. RBCs that sickle more rapidly at a low oxygen tension generate higher slope values, which is favorable for patients. Lower Slope values reflect a heterogeneous population consisting of a percentage of cells already sickled at normoxia and a population of RBCs that start to sickle at a high oxygen tension, which is unfavorable. The parameters provided by oxygen gradient ektacytometry, reflect RBC characteristics that are involved in (the frequency of) vaso-occlusive episodes.[5] Moreover, oxygen gradient ektacytometry-derived parameters have been shown to change significantly as a result of hydroxyurea treatment and transfusion therapy.[5] Other modalities of ektacytometry have shown improvements in RBC deformability upon hydroxyurea treatment.[6,7]

Patient characteristics before and during hydroxyurea treatment are presented in Table 1. Results after 3 months of hydroxyurea therapy were promising with all four oxygen gradient ektacytometry-derived parameters improving substantially. However, after 6 months of hydroxyurea therapy, in both twins a decrease in EI_{max}, EI_{min} and Slope, and an increase in PoS was noted, compared to previous oxygen gradient ektacytometry curves (Figure 1A–E). These changes in parameters were accompanied by a decrease in HbF levels and MCV. The decrease in Slope was accompanied by an increase in red cell distribution width (RDW) indicating that the heterogeneity of the RBC population had increased. After verification of treatment adherence, it was hypothesized that the dosage of hydroxyurea was too low considering the body weight gain of both sisters. Indeed, hydroxyurea was now given at a dosage of 20.24 (Twin 1) and 17.92 (Twin 2) mg/kg. After dose modifications were made, oxygen gradient ektacytometry-derived parameters restored in both twin sisters (Figure 1A–F).

TABLE 1 Laboratory and clinical characteristics before and during hydroxyurea treatment.

Twin 1	Baseline	1 month	3 months	6 months	8 months
Laboratory parameters					
HbF (%)	9.1	14.8	18.3	14.4	22.4
HbS (%)	80.7	74.1	69.8	73.8	64.2
Hb (g/dL)	7.9	7.6	9.6	8.5	8.2
ARC (10^9/L)	688	200	247	445	490
MCV (fl)	91	102	104	99	114
MCHC (g/dL)	37.2	37.1	37.5	33.2	35,7
RDW (% CV)	21,8	22,4	14,5	17,6	17,4
Clinical parameters					
Body weight (kg)	22.3	nd	nd	24.7	nd
BMI (kg/m^2)	15.7	nd	nd	16.3	nd
HU dosage (mg/kg)	22.4	nd	nd	20.2	30.4
Twin 2	**Baseline**	**1 month**	**3 months**	**6 months**	**8 months**
Laboratory parameters					
HbF (%)	7.8	11.8	16.5	9.2	18.0
HbS (%)	82.0	78.1	72.0	82.7	74.1
Hb (g/dL)	7.9	8.2	7.0	8.1	7.9
ARC (10^9/L)	695	315	346	586	422
MCV (fl)	90	100	106	97	111
MCHC (g/dL)	36.7	35.8	35.5	33.3	35,9
RDW (%CV)	22,3	18,9	16,3	20,7	17,8
Clinical parameters					
Body weight (kg)	25.2	nd	nd	27.9	nd
BMI (kg/m^2)	17.0	nd	nd	17.9	nd
HU dosage (mg/kg)	19.8	nd	nd	17.9	nd

Abbreviations: ARC, absolute reticulocyte count; BMI, body mass index; Hb, hemoglobin; HbF, fetal hemoglobin; HbS, hemoglobin S; HU, hydroxyurea; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; nd, not determined; RDW, red cell distribution width.

Our case report shows that oxygen gradient ektacytometry-derived parameters can be used to assess hydroxyurea therapy efficacy and that they aid in the titration of an individual patient's dosings as they change even after subtle adjustments of the dose per kilogram. Despite sufficient evidence on the safety and efficacy of hydroxyurea in sickle cell disease patients, it is still underutilized in clinical practice.[8] The most common provider-related barriers are concerns about patient adherence to therapy and (late) toxicities.[9,10] Oxygen gradient ektacytometry provides visual evidence of the effects of hydroxyurea treatment that can be understood by both patients and healthcare providers. This may help to promote and monitor treatment adherence and is more gratifying than other methods such as pill counts. Because hydroxyurea does not typically have beneficial short-term effects, seeing oxygen gradient ektacytometry-derived parameters change from baseline until optimal dosing is achieved will help patients and families realize that hydroxyurea is effective. In addition, the clinical and laboratory benefits of hydroxyurea are the greatest when escalated to the maximum tolerated dose (MTD). The MTD of hydroxyurea yields an average increase in HbF of 20%–25%, but HbF response is known to vary widely among patients with HbF percentages ranging from 10% to 15% to more than 40% HbF.[11] Moreover, measurement of HbF in blood lysate only reflects the average HbF content across all RBCs. Due to an uneven distribution of HbF among F-cells (HbF-containing RBCs), cell-specific compartmentalization of HbF across RBCs cannot be evaluated by sole measurement of HbF percentage. Flow cytometric quantification of F-cells is another way to determine specific effects of hydroxyurea.[12] Measurement of F-cells is not ideal as the threshold used for detection of F-cells is not the same threshold that defines protection against sickling. A functional assay, such as oxygen gradient ektacytometry, that assesses all RBC characteristics, overcomes these limitations. However, it should be noted that this technique is not available in every laboratory, and its costs will exceed costs of conventional laboratory tests used to monitor patients on hydroxyurea therapy. Our results highlight that substantial changes in functional properties of the red blood cells can occur even with slight dose changes, weight gain, accompanied by minimal changes in MCV and HbF%.

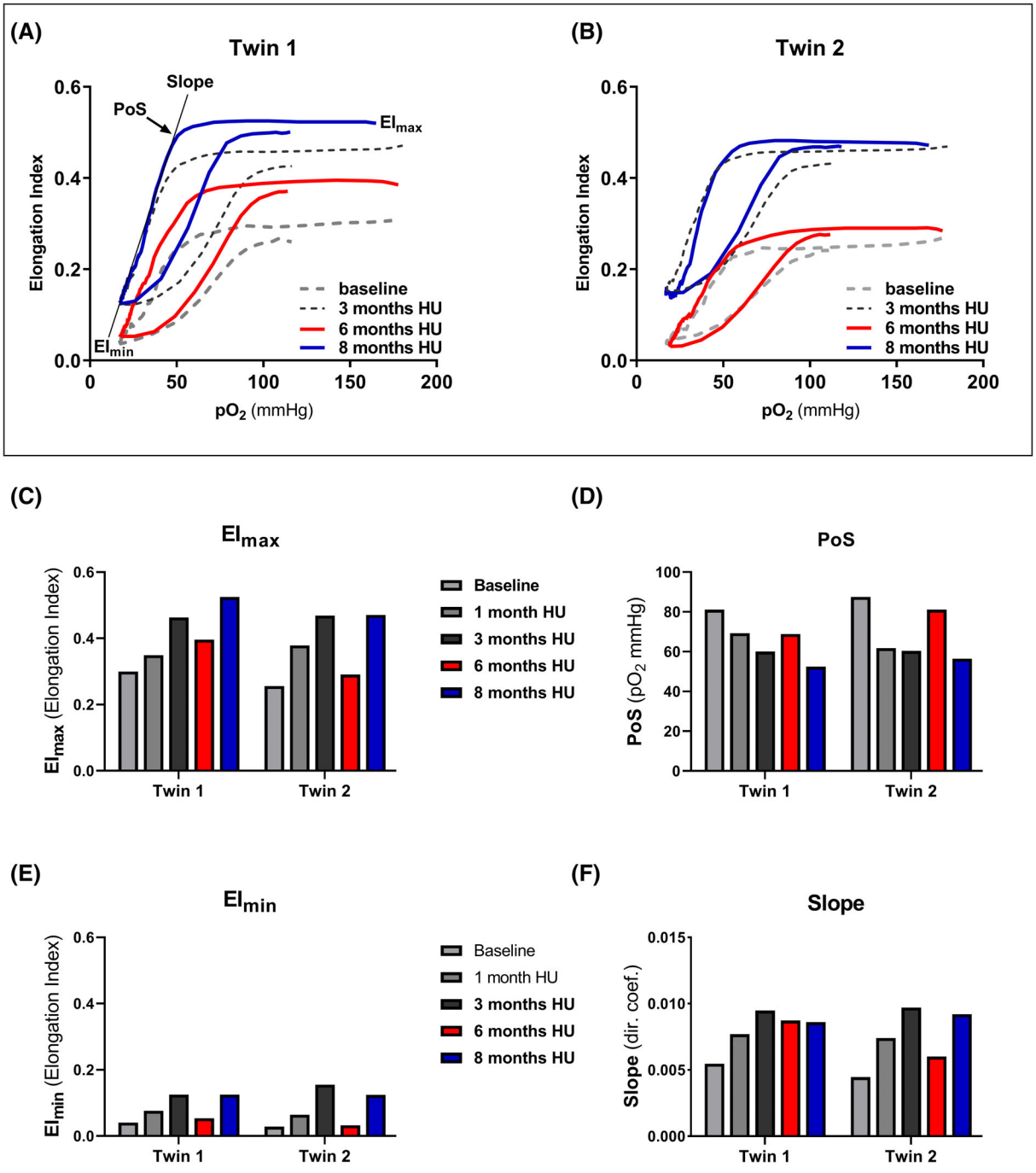

FIGURE 1 Oxygen gradient ektacytometry curves of twin 1 (A) and twin 2 (B) before and during hydroxyurea therapy. (C) Maximum EI at normoxic conditions (EI_{max}) of both twins before and during HU therapy. (D) Specific oxygen tension when 5% decrease of EI is observed (point of sickling [PoS]) of both twins before and during HU therapy. (E) Minimum EI measured at end of deoxygenation (EI_{min}) of both twins before and during HU therapy. (F) Directional coefficient (Slope) of the downward part of the curve after deoxygenation is initiated. This indicates the rapidity of sickling of RBCs upon deoxygenation and is suggested to be a reflection of the heterogeneity of the RBC population. HU, hydroxyurea.

Due to the limited availability of this technique, its use might be limited to specific situations, such as non-responders or when combination therapies are given.

In conclusion, oxygen gradient ektacytometry quantifies RBC deformability and comprehensively assesses various RBC characteristics such as MCV, MCHC, HbF%, and HbS% that together determine sickling behavior as influenced by hydroxyurea medication. Therefore, oxygen gradient ektacytometry curves and its parameters, especially PoS and EI_{min}, visualize how hydroxyurea improves RBC features and decreases sickling tendency. This technique can be used specific situations, to assess non-responders or when combination therapies are given.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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