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# Early detection of response to hydroxyurea therapy in patients with sickle cell anemia.

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## **Abstract**

*Red blood cells and reticulocyte parameters were determined on peripheral blood from a subset of patients enrolled in the Multicenter Study of Hydroxyurea in sickle cell anemia. Multiple blood samples were obtained every two weeks. Cellular indices were measured by flow cytometry. Generalized linear models were used to determine the relationship between the longitudinal trajectories of red blood cell and reticulocyte indices and hydroxyurea usage. There was a significant relationship between hydroxyurea usage and most of the red blood cell and reticulocyte indices. Hydroxyurea produced higher value trajectories than those generated by placebo usage for the hemoglobin content of both the red blood cells and reticulocytes and for the mean corpuscular volume of*

*reticulocytes. These changes were first detected 10 weeks after starting hydroxyurea and before the increase in hemoglobin F levels. The data suggest that subtle and early markers of response to hydroxyurea reside in the hemogram.*

**Key words:** Hydroxyurea, Sickle cell anemia, Reticulocytes, Responders, Indices

## **Introduction**

The salutary effects of hydroxyurea (HU) in sickle cell anemia (SS) include decreased frequency of painful crises, decreased incidence of acute chest syndrome, decreased blood transfusion, and decreased mortality and morbidity (1-5). Markers of the response to HU include increased Hb F level, macrocytosis, improved RBC survival and deformability, and decreased WBC count (6-7). In this study we report markers of early response to HU by using generalized linear mixed models (GLM) to determine the relationship between HU usage and the longitudinal trajectories of RBC indices and the corresponding longitudinal trajectories of reticulocytes indices.

## Methods

RBC and reticulocyte parameters were determined on peripheral blood from selected patients enrolled in the placebo (PL) controlled double blind multicenter study of hydroxyurea (MSH) in SS. The study cohort for this analysis consisted of 21 patients from the Thomas Jefferson University, Cardeza Foundation for Hematologic Research (10 HU subjects, 11 PL subjects). Multiple blood samples were obtained during enrollment in the study at a rate of about once every two weeks. The mean weeks of follow-up from enrollment date was 86.6 weeks (range 10-172 weeks). The RBC/PLT channel of the Siemens H\*3 RTX (H\*3) hematology analyzer measures the laser light scattered at 2 different angles by red cells which have been isovolumetrically sphered. Using the Mie theory of light scatter the low angle ( $2-3^{\circ}$ ) light intensity directly measures RBC volume (MCV, femtoliters) and the high angle ( $5-15^{\circ}$ ) light intensity directly measures refractive index which is a linear function of red cell hemoglobin concentration (CHCM, cell hemoglobin concentration mean, grams per deciliter). The Reticulocyte channel reagent also isovolumetrically spheres as well as selectively staining the reticulocytes with the nucleic acid-binding dye Oxazine 750. Using the same laser optics and principles as the RBC/PLT channel, reticulocyte volume (MCV<sub>r</sub>, femtoliters) and reticulocyte hemoglobin concentration (CHCM<sub>r</sub>, grams per deciliter) are directly measured. An additional detector measures laser light absorption by the Oxazine 750 dye to separate the reticulocytes from the mature red cells. The RBC/PLT channel measures the mean hemoglobin content of the red cells (CH) and the reticulocyte channel measures the mean hemoglobin content of the reticulocytes (CH<sub>r</sub>) by multiplying the volume and the cell hemoglobin concentration

mean. RBC and Reticulocyte indices were measured on a Siemens H\*3 RTX (H\*3) hematology analyzer. Laser light scattering was used to quantify cell volume, Hb concentration, and the light absorbance of cells stained with oxazine 750 to detect reticulocytes and distinguish them from mature cells. A total of 20,000 cells were counted for each sample. After the volume (V) and Hb concentration (HC) of individual mature RBC and reticulocytes were measured, the Hb content (CH) of individual cells was calculated (8).

Generalized linear mixed models (GLM) were used to determine the relationship between the longitudinal trajectories of RBC and reticulocyte indices and HU usage (9-12). A first-order autoregressive structure was used (1). Information criteria [Akaike Information Criterion (AIC)] (13), Akaike Information Criterion, Small Sample Size Corrected Version (AICC) (14) and the Bayesian Information Criterion (BIC) (15) were used to compare and determine which GLM provided better insight into the relationship between the longitudinal trajectories of RBC and reticulocyte indices and HU usage. The information criteria are all in “smaller-is-better” form. Since the GLM considered longitudinal data, the time at which the longitudinal trajectories were influenced by HU usage could also be determined. PROC MIXED (SAS 9.1) was used for the GLM analyses. Statistical significance was defined as a 2-sided p-value <.05.



## Results

The mean weeks (wks) of follow-up from study enrollment date was 86.6 wks (standard deviation (sd)=38.84 wks; max=172 wks, median=84 wks, min=10 wks). The mean age of this patient cohort at the start of follow-up was 29.0 years (SD =4 .77 years; max=37 years, median=30 years, min=19 years). There were 13 males (61.9%) and 8 females (38.1%).

Goodness-of-fit criteria for the GLM indicated that the RBC indices modeled more precisely than those of reticulocytes. However, both the RBC indices and reticulocytes provided useful information regarding the relationship between their longitudinal trajectories and HU usage (Table 1).

There was a significant relationship between HU usage and the following longitudinal trajectories: mean cell hemoglobin (CH) ( $p < .0001$ ), reticulocyte mean cell hemoglobin (CHr) ( $p < .0001$ ); Cell hemoglobin concentration mean (CHCM) ( $p = .0394$ ); Reticulocyte mean cell volume (MCVr) ( $p = .0019$ ); hemoglobin distribution width (HDW) ( $p = .0003$ ) and red blood cell volume distribution width (RDW) ( $p = .0008$ ). HU usage produced higher value trajectories than those generated by placebo usage for the following: CH, CHr; and MCVr (Fig. 1). These changes were first detected 10 weeks after starting HU and before the increase in Hb F levels.

## Discussion

Although HU has been proven to have salutary effects in patients with SS (1-3) its utilization continues to suffer from two major drawbacks. These include its underutilization (16) and the fact that at least 25% of patients who take it are non-responders to its use (1, 2). These two drawbacks seem to re-enforce each other. Thus some patients may be falsely labeled as non-responders if no effect on frequency of painful crises and/or no increase in the level of Hb F is seen within 2-3 months after starting HU at a dose of 10-15 mg/kg/day. The reason why some patients do not respond to HU is unknown. One possibility is that it fails to recruit dormant bone marrow fetal stem cells that are capable to produce fetal Hb. Moreover, it usually takes at least six months of therapy with HU before an increase in Hb F is seen (7). Thus an early sign of a response-in-progress to HU therapy is important information for the provider who is monitoring the the clinical and hematological data of patients after the initiation of therapy with HU.

In this study we report markers of early response to HU by using generalized linear mixed models (GLM) to determine the relationship between HU usage and the longitudinal trajectories of RBC parameters and the corresponding longitudinal trajectories of reticulocytes parameters. Specifically HU therapy showed significant changes in the Hb content (CHr) and cell volume of reticulocyte (MCVr) compared to placebo within 10 weeks after starting HU indicating an early sign of response to therapy.

Thus the sequence of signs of response to HU include increase in reticulocyte indices (CHr and MCVr) within 10 weeks after therapy followed by increase in RBC indices and a later increase (up to six months) in Hb F level.

## **Conclusion**

Together the data suggest that subtle and early markers of response to HU reside in the hemogram. This is a fast, simple and inexpensive methodology provided the lab in question has the capability to determine the indices of both reticulocytes and RBC.

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**Legend to Figure 1**

Time trends in MCV and MCVr by treatment assignment