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POSTER ABSTRACTS

114.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: CLINICAL AND EPIDEMIOLOGICAL

A Novel, Rapid, and Accurate Quantitative Hydroxyurea Assay

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Introduction: Hydroxyurea is a highly effective treatment for children and adults with sickle cell anemia (SCA) and is now considered standard-of-care therapy in high-resource countries. There is wide interpatient variability in the optimal dose, which is defined as the daily dose that provides maximal induction of protective fetal hemoglobin to decrease erythrocyte sickling while causing only mild marrow suppression. The time and cost of hydroxyurea dose titration are barriers to its widespread use in low-resource settings. The titration process required to identify an optimal dose can be abbreviated by measuring individualized hydroxyurea pharmacokinetics (PK) to guide personalized precision dosing, which could be especially useful in low-resources settings with limited laboratory monitoring. However, measurement of hydroxyurea in serum and other physiological fluids is not commonly performed during routine clinical treatment. Current methodology to quantify hydroxyurea concentrations requires complex and time-consuming chemical derivatization followed by detection by colorimetric spectrophotometry, high performance liquid chromatography (HPLC), or liquid chromatography mass spectrometry (LC-MS). To consider performing PK-guided dosing in low-resource settings, a simple, accurate, and efficient hydroxyurea assay is needed.
Methods: A commercially available open-system HPLC unit for hemoglobin separation (SmartLifeLC, PolyLC Inc, Columbia MD) was equipped with a 3.5-micron, 4.6 mm × 50 mm Zorbax Eclipse XDB-C18 column with matching guard column (Agilent Technologies, Santa Clara, CA); mobile phases of 46% methanol and 100% acetonitrile; and a 200-600nm multi-wavelength detector. The chemical derivatization process was modified from previously published techniques that use xanthydrol (Sigma-Aldrich, St. Louis MO) for detection of primary amides. N-methylurea (Sigma-Aldrich) was included as an internal standard. Hydroxyurea (Sigma-Aldrich) was diluted in whole blood collected from healthy controls to create a calibration curve encompassing the typical pharmacological range of hydroxyurea at 80, 40, 20, 10, and 5 mg/mL. Quality Control (QC) samples were prepared at 50, 25, 12.5, and 6.25 mg/mL to validate the calibration curve before testing patient samples. Blood was applied to 20mL volumetric absorptive microsampling devices (Mitra, Trajan Scientific and Medical, Torrance CA) and dried for future elution and analysis. HPLC results were compared to LC-MS results to assess accuracy.
Results: Blood was eluted from the Mitra microsampling device and derivatized in a xanthydrol solution before HPLC analysis. Using a 10-minute run with 210nm wavelength detection, the xanthydrol-modified hydroxyurea reproducibly yielded a linear calibration curve with $r^2 > 0.95$ (Figure), and the actual concentrations were within 20% of the theoretical values. Repeated testing of control and patient samples revealed a typical intra-day coefficient of variation <15% and inter-day coefficient of variation <20%. The agreement between nominal values and assay results determined for a wide range of QC samples from both methods exceeded the FDA-recommended threshold of 67% for chromatographic assay validation with 86% agreement on LC-MS and 85% with the new method. The entire elution and derivatization procedure could be completed using dried Mitra blood samples in less than 60 minutes.
Conclusions: Quantitative hydroxyurea measurement of blood or serum can now be accomplished using a novel, rapid, HPLC technique that features xanthydrol-based derivatization of hydroxyurea, an internal standard, and UV wavelength detection. This technique is accurate, reproducible, and relatively simple, and thus deemed suitable for hydroxyurea concentration measurements needed for PK-guided dosing calculations for children with SCA living in low-resource settings. This novel technique will be added to the multi-country Realizing Effectiveness Across Continents with Hydroxyurea (REACH, NCT01966731) trial that will feature PK-guided precision dosing for young children with SCA starting hydroxyurea treatment.

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Figure

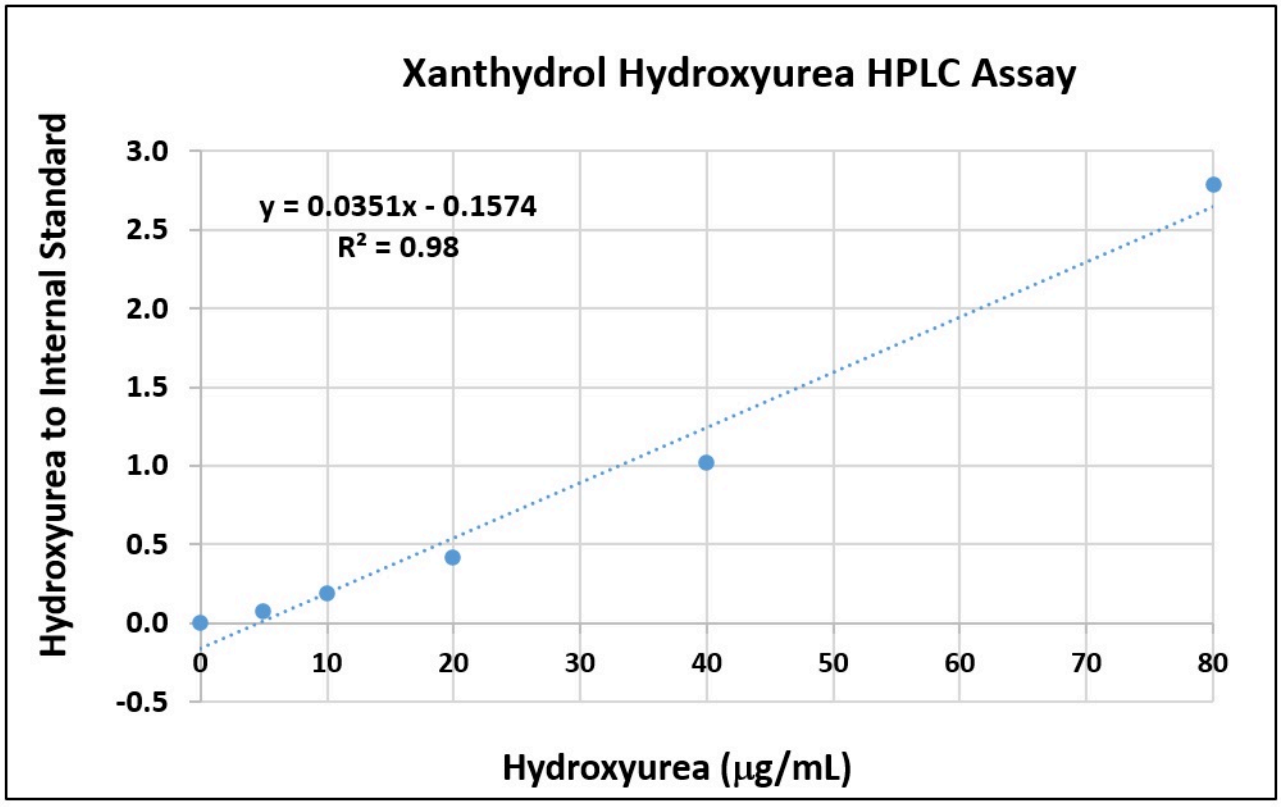

Figure 1

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