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POSTER ABSTRACTS

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

Biologic Assessment of RBC Biology and Neutrophil Activation: Correlation with Sickle Cell Disease Activity

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Sickle cell disease (SCD) has pleiomorphic effects on affected individuals, most typically including both hemolysis and vaso-occlusive events (VOE). However, previous reports have suggested that these two paradigmatic presentations of SCD represent two poles of a spectrum of disease activity, so that patients with the highest frequency of VOEs are at one end, and patients with high hemolytic rates and organ damage, such as nephropathy and pulmonary hypertension, are at the other end.

We performed an observational study of erythroid (RBC) factors, neutrophil activation, and clinical parameters, including vaso-occlusion event (VOE) frequency, in order to determine which factors were related to each other and to either hemolytic rate or VOE frequency. 115 patients in steady state, at least 3 weeks after last VOE, with either HbSS, HbSß $^{+/0}$ thalassemia, or HbS/HPFH, were enrolled from 10/20/2020 - 10/25/2022. Erythrocytes and plasma were isolated from whole blood and analyzed for RBC deformability, intracellular RBC [ATP], RBC phosphatidylserine exposure, and plasma matrix metallopeptidase 9 (MMP9), a product of neutrophil activation and degranulation. A subset of samples were examined for RBC reactive oxygen species (ROS) content and adhesivity to laminin with and without stimulation by epinephrine. Clinical data collected included complete blood count parameters, LDH, total bilirubin, reticulocyte (retic) counts, and health care utilization for VOE in the preceding year. Deformability was measured as maximal elongation index (EImax) at Pa=30 by laser optical rotational red cell analyzer (Lorrca). RBC ROS was quantitated using 2′,7′-dichlorofluorescein and expressed as nM/mg Hb. MMP9 was measured by commercial ELISA assay.

We found (N=102 with complete data) that EImax was directly proportional to total [Hb] ($P<0.0001$), irrespective of gender. Higher EImax was also associated with lower parameters of hemolysis ($P = <0.0001$, 0.0002, 0.0004, and <0.0001 for total LDH, total bilirubin, absolute retic count, and % retics, respectively). Higher HbF levels were associated with greater deformability ($P<0.0001$), and RBC deformability was inversely associated with PS exposure ($P<0.0001$) and RBC ROS content ($P=0.0484$, $N=23$). Surprisingly, however, greater deformability was not associated with higher RBC ATP content or lower health care utilization for VOE, while there was only a trend toward an inverse association with plasma MMP9 ($P=0.0771$). Interestingly, we also found that RBC [ATP] was lower in the RBCs of patients with higher total Hb, while it was higher in individuals with higher retic counts, suggesting that more long-lived RBCs have diminished [ATP], despite the fact that both [ATP] and total Hb have been reported to rise substantially after treatment with pyruvate kinase activators etavopivat and mitapivat. Higher ATP was also not significantly associated with a decrease in RBC adhesivity.

Of note, higher HbF levels were significantly associated with lower hospital admissions for VOE ($P=0.0053$), although not with frequency of out-patient health care utilization for VOE (ED and day hospital visits). HbF levels also showed a trend toward inverse association with plasma MMP9 ($P=0.0613$).

These results may help explain why therapeutic agents that increase total Hb by shifting the oxygen dissociation curve, thereby reducing sickling and hemolysis, appear to have little or no effect on VOE rate. Similarly, senicapoc, which also reduces hemolysis, although by a different mechanism, failed to lessen VOE frequency. We conclude that RBC deformability, PS exposure, and RBC ROS are markers associated with the hemolytic subphenotype but not with the somewhat overlapping but divergent adhesiveness and frequent VOE subphenotype. Anti-adhesive agents (crizanlizumab, rivipansel, sevuparin) have thus far failed to yield marked improvements in VOE frequency, but our results suggest that, in order to decrease the frequency of VOEs, it is not sufficient to reduce sickling and hemolysis. On the other hand, higher HbF levels were associated with lower admission rates for VOE and a trend toward lower levels of neutrophil activation. This suggests that higher HbF, beyond its ability to reduce sickling, also alters other biological characteristics of SS RBCs and is accompanied by reduction in the inflammatory nature of SCD, thereby contributing to a decrease in VOE frequency.

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