Paper # 2028

PREVALENCE AND RISK FACTORS FOR PULMONARY EMBOLISM IN PEDIATRIC SICKLE CELL DISEASE.

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Background: Patients with sickle cell disease (SCD) have a high risk for venous thromboembolism (VTE). An adult SCD study identified higher rates of pulmonary embolism (PE) (0.44%) as compared to age and race-matched controls (0.12%) (1). In the general pediatric population, the estimated PE rates are 0.058-0.064% (2). Slightly over half of pediatric PE cases are associated with a deep vein thrombosis (DVT) (3). Studies examining prevalence and risk factors for PE in children with SCD are lacking.

Objectives: Describe the prevalence of PE in children with SCD and identify potential risk factors associated with PE using a nationwide administrative claims data from Pediatric Health Information System (PHIS).

Design/Method: Children with SCD between 0-21 years of age from January 2010 to June 2021 were included. PE was identified using International Classification of Diseases (ICD)-9 or 10 codes and confirmed with documentation of either an anticoagulant or PE-related imaging study. SCD, DVT and risk factors such as obesity, pregnancy, acute chest syndrome (ACS), Central nervous system (CNS) vasculopathy were identified using ICD codes. Billing codes were used to identify presence of central venous line (CVL), apheresis, use of hormonal therapy, anticoagulants and hydroxyurea. Logistic regression analysis was performed to assess association between risk factors and PE.

Results: We identified 22,631 unique patients with SCD with a median age of 10.8 years (range: <0.1-20.9) from PHIS. A total of 120 (0.53%) patients developed a PE with median age of 17.4 years (range: 6.6-20.9). A concurrent diagnosis of ACS was documented in 58% of patients with PE. Prior history of CVL, recurrent ACS (> 1 episode prior to PE diagnosis), hydroxyurea use, older age, CNS vasculopathy, apheresis, number of total hospital and ICU admissions were significantly associated with PE on bivariate logistic regression analysis. The diagnosis of DVT was significantly more common among patients with admissions for PE compared to those without PE (28% vs 0.6%; p<.0001).

Conclusion: The prevalence of PE in children with SCD is 0.53%. Over half of these patients had ACS at time of diagnosis of PE (58%) and 28% had DVT. Factors significantly associated with PE such as prior history of CVL, recurrent ACS, use of hydroxyurea, older age, CNS vasculopathy, apheresis and number of total number of hospitalizations suggest that the risk for PE in SCD is related to the severity of disease state.

References

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Paper # 2029

THE EFFECT OF HEMOGLOBIN ON OXIDATION OF HMGB1 AND PLATELET ACTIVATION

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Background: Inflammation plays a key role in propagating the pathogenesis of hemolytic diseases. Patients with elevated cell-free hemoglobin (Hb) are at significantly higher risk of thrombosis, which can lead to life-threatening complications such as pulmonary emboli and stroke. On a cellular level, it is well established that in hemolytic states, platelet activation is elevated and correlates with hemolysis, and that platelet activation stimulated by Hb is driven by mitochondrial reactive oxygen species (mtROS) generation. However, the contribution of inflammatory signaling to platelet activation and its cross-talk with hemolysis remain unknown. High-mobility group box 1 (HMGB1), an inflammatory mediator released by cells, has been shown to be elevated in sterile inflammatory conditions and to stimulate platelet activation. Notably, the ability of HMGB1 to mediate platelet activation is dependent on the oxidation state of critical cysteine residues in the protein.

Objectives: We hypothesize that in hemolytic conditions, Hb and its derivatives oxidize HMGB1 to increase platelet mtROS production leading to platelet activation.

Design/Method: Recombinant HMGB1 was incubated in vitro with metheme derived from Hb and quantified by spectroscopy. Carbonyl groups attached to protein side chains on HMGB1 were derivatized to 2,4-dinitrophenylhydrazone (DNP-hydrazone) and quantified by Western blot immunodetection. Protein oxidation of HMGB1 was quantified by signal intensity of DNP ab (OxyBlot protein oxidation detection kit, Millipore) co-localized to signal from anti-HMGB1 ab (Biolegend). Platelets were isolated from the whole blood samples of human participants, and platelet activation was measured by flow cytometry using PE antibody to GPIIb (CD41) to mark platelets and APC to detect exposure of surface CD62P (p-selectin) upon activation. Platelet mtROS were estimated using MitoSOX Red and fluorescence spectroscopy. Data was analyzed using FlowJo software and nonparametric statistical tests.

Results: We found that exposure of HMGB1 to metheme produced a 4-fold increase in the oxidation signal of HMGB1 compared with untreated HMGB1. Additionally, the presence of cell-free Hb in solution with HMGB1 significantly enhanced HMGB1-mediated platelet activation in a dose-dependent manner as the concentration of Hb increased (P=0.0211). We confirmed that treatment of isolated human platelets with Hb or with HMGB1 each independently increased platelet mtROS production by 2-fold (P=0.0004) and 4-fold (P=0.0008), respectively. Scavenging mtROS using MitoTEMPO attenuated platelet activation by HMGB1 +

Hb by 2-fold.

Conclusion: Together, these studies suggest a mechanism of synergy between hemolysis and inflammatory and redox signaling in potentiating platelet-driven thrombosis.

Paper # 2030

STANDARDIZED THROMBOPROPHYLAXIS PROTOCOL IN MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN

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Background: Patients with Multisystem Inflammatory Syndrome in Children (MIS-C) exhibit laboratory evidence of hypercoagulability and are at risk of experiencing thrombotic complications. We developed and implemented a standardized multidisciplinary treatment approach which encompassed a thromboprophylaxis protocol and a computerized clinical decision support system including a provider order set to guide and unify practice. In high-risk patients defined as critically ill and/or having additional risk factors for thromboembolism, prophylactic-dose enoxaparin (target anti-Factor Xa of 0.1–0.3 U/mL) was added.

Objectives: To evaluate impact of implementation of a standardized thromboprophylaxis protocol in hospitalized patients with MIS-C.

Design/Method: We conducted a retrospective study of patients who were admitted to our center between March 2020 and December 2021 with confirmed MIS-C based on Centers for Disease Control and Prevention case definition. Relevant data were extracted from prospectively maintained institutional databases and the electronic medical records and were summarized using descriptive statistics. Key outcome measures included frequency of objectively confirmed venous and/or arterial TE during hospitalization and within 30 days after discharge and frequency of major bleeding and/or clinically relevant nonmajor bleeding (CRNMB) defined according to the International Society on Thrombosis and Haemostasis criteria.

Results: A total of 136 patients (59 females, median age 8 years) with confirmed MIS-C were included in this study. Forty-five patients (33%) were ≥12 years of age. Of 136 patients, 124 patients (91%) required intensive care unit (ICU) stay and 64 patients (47%) required a central venous catheter for a median duration of 5 days [Interquartile range (IQR) 4-7]. The median total hospital and ICU length of stays were 11 days [IQR 6-14] and 3 days [IQR 2-6], respectively. Prophylactic-dose enoxaparin was initiated in 119 patients (88%) who were deemed high-risk per our protocol at a median of 1 day after admission [IQR 0-3] achieving target levels at a median of 1 day [IQR 1-2]. The median first anti-Factor Xa level was 0.13 u/mL [IQR 0.05-0.19]. Only 1 patient (0.7%) developed symptomatic non-catheter related superficial vein thrombosis requiring therapeutic anticoagulation. There were no other TEs encountered in our cohort. Bleeding events occurred in 5 patients (4.2%). All bleeding events were considered

CRNMB (gastrointestinal bleeding in 4 patients and epistaxis in 1 patient). There were no mortalities.

Conclusion: Implementation of an institutional standardized thromboprophylaxis protocol in patients with MIS-C was feasible and led to timely initiation of prophylactic anticoagulation and low rates of TEs and bleeding complications.

Paper # 2031

CRISPR TARGETING OF SBDS IN MAMMALIAN CELL LINES REVEAL INCREASED TP53/CDKN1A AND SLOW GROWTH

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Background: Shwachman-Diamond syndrome (SDS) is an inherited bone marrow failure syndrome associated with a significant risk for transformation to myeloid neoplasia. Biallelic mutations in the Shwachman-Bodian-Diamond-Syndrome (*SBDS*) gene account for 90% of cases and result in impaired ribosomal assembly. The molecular pathogenesis of SDS and its transformation to neoplasia in some patients is poorly understood. Based on RNA-Seq and western blotting analysis in our zebrafish model of SDS (*JCI Insight*, 2020), we hypothesize that stress responses that lead to metabolic reprogramming cause the syndrome and its neoplastic complication.

Objectives: Characterize the biochemical pathways involved in pathogenesis of SDS using CRISPR-Cas9 edited *SBDS* mammalian cell lines.

Design/Method: We created *SBDS* knockdown HeLa cell line using CRISPR genome editing. We designed our guide RNA to create indels in exon 2, which would approximate the K62X truncations in *SBDS*. We analyzed the expression of *TP53* pathway markers and lipid metabolism markers in the edited cell lines using real-time polymerase chain reaction (RT-PCR).

Results: While we did not identify any clones with complete knockout of *SBDS*, we did isolate clones with less than 10% protein expression. We correlated decreased *SBDS* protein expression with a 3-fold decrease in cellular growth rate. Doubling time was 36 hours in the *SBDS* knockdown clones compared to 22 hours in parent cell. *TP53* showed a 4-fold increase while *CDKN1A* showed a 12-fold increase in the clone with 90% reduction of *SBDS* relative to parental cells. We observed a 2.5-fold increase in *BAX,* and corresponding decrease in MDM2. These results were similar to those in the zebrafish KO model published by our group in 2020. Interestingly, unlike the *sbds* $^{-/-}$ zebrafish, we observed a 2-fold decrease in *FASN, PPARG* and *SREBP1* in the clones relative to the parental cell.

Conclusion: To our knowledge, we generated the first mammalian cell line deficient in *SBDS* based on CRISPR. Levels of *CDKN1A* and *TP53* were significantly increased

in *SBDS* knockdown clones, which would lead to cell cycle arrest and slow growth as observed in these clones. We found a decrease in markers of lipid metabolism, which could be due to lack of fatty acids in the tissue culture media, unlike zebrafish which are fed a lipid-rich diet. We are currently creating other mammalian cell lines using our CRISPR /Cas9-mediated genome editing model, which can be used to provide new insights into the pathogenesis of SDS and validate biochemical findings in other experimental systems such as zebrafish and yeast.