Patients with sickle cell disease (SCD) show various manifestations of arterial and venous thrombosis, especially around sickle cell crises. There are numerous pathophysiologic factors that could explain hypercoagulability in SCD (vessel injury, enhanced platelet function, impaired fibrinolysis, activated coagulation cascade, etc). The aim of this study is to demonstrate the association of acquired protein S deficiency (PSD) with SCD, elucidate the mechanism in vitro, and correlate it with timing of sickle cell crises in our patient population. This study includes every patient diagnosed with sickle cell trait (SCT) and SCD in a large center of SCD excellence between 2000 and 2017. The data are gathered using a user-friendly interactive software developed in-house for the evaluation of health care quality, efficiency, and effectiveness. The EMR database (EPIC) we use consists of laboratory, clinical, pathological, and radiological reports allowing us to use different search criteria and to outline cutoff values for continuous and categorical variables while permitting us to define a time frame for the different observations. It is a useful resource to compare and contrast how two or more populations are affected by a particular disease and to separate out patients with a particular diagnosis. The initial data we extracted looked at patients with a diagnosis of SCD between 2000 and 2017 who had acquired PSD (<55%) and compared it to patients who have SCT with acquired PSD (<55%) in the same time period. We observed that out of 5,799 patients who had a diagnosis of SCD, 74 patients (1.27%) had a concomitant PSD, and out of 11,551 patients with SCT, 110 patients (0.95%) had a concomitant PSD. These preliminary results suggest a strong association of acquired PSD with SCD when compared to the general population (incidence of 0.2%) and, more specifically, the role of sickled red blood cells in acquired PSD. A chi-square analysis of SCD vs SCT ($P = .04$) and SCD vs general population ($P = .03$) showed a statistically significant increase in the incidence of PSD in SCD > SCT > general population. The higher incidence of PSD in SCD compared to SCT and the general population suggests that PSD is directly dependent on the extent of sickled cells. We hypothesize that acquired PSD in SCD is due to direct binding of protein S to sickling red cells that express increased surface phosphatidylserine. We are currently evaluating this hypothesis by in vitro flow cytometry studies. We are also studying how acquired PSD correlates with the timing of sickle cell crises in our patient population. In summary, acquired PSD may be another risk factor that contributes to thrombosis in SCD and thus may be a useful clinical marker to predict and/or monitor thrombosis in SCD.

### In Situ Multiplex Immunofluorescence Analysis of Plasma Cell Myeloma Tissue Sections

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Plasma cell myeloma (PCM), with a highly heterogeneous clinical behavior and response to therapy, accounts for approximately 10% of hematologic malignancies. Current diagnostic and prognostic tests include serum-based assays, multiparametric flow cytometric analysis of bone marrow aspirates, morphologic and immunohistochemical analysis of bone marrow sections, cytogenetic analysis, and in vivo imaging. None of these tools allow for determination of expression of large numbers of antigens on a single myeloma cell in spatial context, which may be useful in order to better understand disease progression and therapy response. In a pilot study, we sought to evaluate the performance of a new in situ hyperplex immunofluorescence platform, Cell-DIVE, that allows spatial and subcellular localization of over 60 markers on three bone marrow samples (two non-decalcified clot sections and one decalcified core biopsy) from PCM cases. The platform utilizes a signal cycling process wherein repeated cycles of staining with fluorophore-conjugated antibodies, imaging, and signal removal are performed on the same tissue sections. Postcycling, images from multiple cycles are registered, corrected for tissue autofluorescence, and segmented into single cells and subcellular compartments using compartment-specific markers. To demonstrate feasibility, a panel of five markers that included plasma cell markers (CD138, CD45), markers of clonality (kappa and lambda light chains), and the proliferation marker Ki-67 were evaluated on a single histologic section. A virtual H&E image was also generated from the same tissue section. Both clot and core biopsy samples showed staining patterns similar to immunohistochemistry—the PCM cells expressed CD138 and either kappa or lambda light chains but lacked CD45. The cellular localization of staining was also as expected, with CD138 displaying surface staining, light chains displaying cytoplasmic staining, and Ki-67 displaying nuclear staining. This new immunofluorescence platform blends the strengths of immunohistochemistry (preservation of tissue architecture) with those of flow cytometry (analysis of many antigens on the same cell). Our preliminary data suggest that the in situ hyperplex immunofluorescence platform is a powerful new approach to study PCM biology, which allows for preservation of the spatial context.
of malignant plasma cells relative to a heterogeneous tumor microenvironment, assessing more antigens on a single cell than has been possible previously. Additionally, this platform gives us a new method for assessing heterogeneity at the level of the tumor cell clone—an important research tool that shows great promise for synergizing with other newly emerging, single-cell-based technologies.

Early Detection of Doxorubicin-Induced Cardiotoxicity With High-Sensitivity Troponin T in Chemotherapy-Treated Patients

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Detection of chemotherapy-induced cardiotoxicity has historically relied on clinical presentation and cardiac imaging measures. Recently, global longitudinal peak systolic strain (GLS) measures with speckle tracking echocardiography (STE) and high-sensitivity troponin T (hs-TnT) have been utilized to evaluate the development of cardiotoxicity. The increased sensitivity of these methods may allow us to detect early development of cardiotoxicity and predict future cardiac dysfunction in chemotherapy-treated patients. We investigated the effectiveness of hs-TnT and GLS in detecting doxorubicin-induced cardiotoxicity.

Thirty-six patients with newly diagnosed sarcoma were assigned to receive a 72-hour doxorubicin infusion. hs-TnT was monitored before and at 72 hours of each chemotherapy cycle. All samples were assayed at the same time using hs-TnT (Roche Diagnostics). Elevated troponin was defined as hs-TnT >5 ng/L. STE was performed pretreatment, after cycle 3, and at the end of chemotherapy. Only patients who received ≥150 mg/m² of doxorubicin and had at least two STEs were included for evaluation of GLS and left ventricular ejection fraction (LVEF). Six patients (25%) developed cardiotoxicity. The absolute levels of hs-TnT had significantly peaked from precycle baseline, increased starting at cycle 2, subsequently in each precycle and during the cycle of therapy (P < .05). Fold changes over baseline hs-TnT level were also significantly increased. In all six patients with cardiotoxicity, GLS increased significantly at the end of chemotherapy, compared with baseline (~21 ± 2 vs –19 ± 2). The increases in GLS by 15% and hs-TnT by 5 ng/L were independent predictors of the development of cardiotoxicity at the end of chemotherapy (P < .05). In conclusion, hs-TnT and GLS predict the development of cardiotoxicity in patients treated with doxorubicin. These two parameters may be useful in predicting and detecting the development of chemotherapy-induced cardiotoxicity and thus reduction of the incidence of its associated morbidity and mortality.

Applying PLASMIC Scoring to Help Diagnose Thrombotic Thrombocytopenic Purpura (TTP)

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Thrombotic thrombocytopenic purpura (TTP) is a rare and serious thrombotic microangiopathy (TMA) syndrome caused by a lack of ADAMTS13 activity, resulting in microthrombi, precipitating end-organ injury and possible death if not treated with plasma exchange. However, there are three challenges in making the diagnosis of TTP: the relatively nonspecific and often subtle constellation of signs and symptoms, the prolonged turnaround time of ADAMTS13 testing, and the infrequency of the disease (~3 cases per million/year).

Case: A previously healthy man in his late 70s presented with acute headache, dizziness, confusion, left upper quadrant abdominal pain, and a 1-year history of intermittent dizziness and headache. Testing revealed a platelet count of 20 × 1,000/µL, creatinine of 0.76 mg/dL, LDH of 1,012 U/L (reference, 117–224 U/L), indirect bilirubin of 1.1 mg/dL (0.2–1.0 mg/dL), undetectable haptoglobin, normal coagulation studies including a normal fibrinogen of 387 mg/dL, and hematocrit of 27%. A peripheral smear showed slight schistocytosis. Imaging studies showed subacute ischemic stroke. Although the patient’s laboratory results showed evidence of microangiopathic hemolytic anemia and low platelets, the positive direct antiglobulin test (DAT), unremarkable physical exam, and a consult service’s impression that “he didn’t look sick enough to have TTP” were confounding the TTP diagnosis. An ADAMTS13 activity level was sent out and the clinical team favored a diagnosis of autoimmune hemolytic anemia with thrombocytopenia over TTP.

Results: The transfusion service calculated the patient’s PLASMIC score using a scoring system recently introduced to rapidly assess and predict the likelihood of severe ADAMTS13 deficiency. The patient met six out of seven criteria, signifying a 62% to 82% probability of having an ADAMTS13 activity level <10%. Due to a differing preferred diagnosis between services, a compromise was reached where rituximab was given to treat a potential autoimmune anemia, and plasmapheresis was started the next afternoon. The ADAMTS13 level was ultimately reported as <5%, consistent with TTP. Following the second exchange treatment, the patient’s ADAMTS13 activity increased to 76%. Following the fourth exchange, his platelet count had increased from 20 to 172 × 1,000/µL, and the LDH decreased from 1,012 to 310 U/L. The patient reported that all of his pain resolved with treatment. Plasmapheresis continued for a total of five treatments, until the platelet count had been >150 × 1,000/µL for at least 3 consecutive days.