labratory received tremendous amount of tests, and here we examined demographic and laboratory data, as well as trajectories of laboratory results, in order to determine the relation between these laboratory parameters, in particular tests of coagulation, to illness severity and mortality. **Methods**: This is a retrospective study of all positive COVID cases who were admitted between 2/22/2020-4/20/2020 at Montefiore Health System (MHS), a large tertiary care center in the Bronx. Together the ambulatory and hospital networks care for 2.8 million visits a year. All adults with positive COVID tests performed by MHS and who were admitted between 2/22/2020-4/20/2020 are considered. All hospitalized COVID positive cases were queried from the electronic medical record system. Physiological, demographic (age, sex, socioeconomic status and self-reported race and/or ethnicity) and laboratory data was captured. A subset of cases were chart-reviewed for accuracy and additional information. Statistical analysis was performed using R studio. **Results**: Discharge from hospital and mortality were the primary measured outcomes. 7096 patients tested positive for COVID, of which 2897 had an associated inpatient admission and 845 patients were seen in the ER and then discharged. A total of 767 COVID positive patients died during hospitalization. A multivariable logistic regression analysis shows increased odds ratio for mortality by age, gender(males > females), BMI, neutrophil to lymphocyte ratio, Charlson Score, and D-Dimer. The receiver operating characteristic curve (ROC) of D-Dimer combined with age showed an area under the curve (AUC) of 0.77. The optimal cut-point, calculated using Youden's index, for the initial D-Dimer to predict mortality was found to be 2.43ug/ml. D-Dimer trajectories between survivors and non-survivors showed a clear separation for non-survivors since admission. **Conclusions:** In this study we comprehensively studied demographic, physiological and laboratory parameters of COVID19+ minority patients in the Bronx, NYC, USA. This study confirms laboratory and clinical observations made by Wuhan studies of COVID19 infected patients. In particular the association of initial D-Dimer and its trajectory during hospitalization with mortality.

Development and Validation of a Liquid Chromatography Mass Spectrometry Method for Simultaneous Measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, Vitamin A, α-Tocopherol, and γ-Tocopherol

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Background: Circulatory fat-soluble vitamin levels are commonly measured to identify deficiencies that may lead to rickets, osteomalacia, night blindness, and reversible motor and sensory neuropathies. We developed and validated a rapid and robust LC-MS/MS method that simultaneously measures 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.
Method: 100 µL of serum was mixed with isotope-labeled internal standard and extracted using a 96-well supported-liquid extraction plate with 1.5 mL of hexanes/isopropanol (90/10) (v/v). Dried eluate was reconstituted with 100 µL of methanol/water (90/10) (v/v) and analyzed by LC-MS/MS with a 10-minute gradient. Accuracy was assessed using NIST Standard Reference Materials SRM972a and SRM968f, patient comparison analysis with a LC-MS/MS method at a reference lab, and spike-recovery studies using patient sera and vitamin D-depleted serum. Analytical measurement range (AMR) was determined by spiking 6 analytes into vitamin D-depleted serum to give 7 specimens at varying concentrations. The lower limit of the measuring interval (LLMI) was assessed using 6 pooled specimens with varying low concentrations of each analyte over 20 days. Precision (repeatability and reproducibility) was assessed using quality control materials. Interference studies were performed using pooled patient specimens spiked with varying concentrations of hemoglobin, bilirubin, or intralipid. Matrix effect was assessed by post-column infusion and by matrix dilution with saline.
Results: The method was linear covering physiological concentrations with $r^2 > 0.99$. Repeatability and reproducibility were <15% CV at all QC levels. LLMI for 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol were 4 ng/mL (15% CV), 4 ng/mL (15% CV), 4 ng/mL (18% CV), 1 µg/dL (20% CV), 0.2 µg/mL (20% CV), and 0.2 µg/mL (8% CV). Recoveries for NIST Standard Reference Materials were between 92 - 111% and between 81 - 122% for spike-recovery studies. Passing-Bablok analyses for vitamin D total, vitamin A, and α-tocopherol demonstrated slopes between 1.04 and 1.11 and r^2 between 0.94 and 0.96. Minimal matrix effect was observed.
Conclusions: We have developed and validated a comprehensive and rapid LC-MS/MS method for the simultaneous measurement of 25-OH D3, epi-25-OH D3, 25-OH D2, vitamin A, α-tocopherol, and γ-tocopherol for clinical use.

Low ADAMTS13 Activity Correlates with Increased Mortality in COVID-19 Patients

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Systemic inflammation and coagulopathy are characteristic hallmarks of COVID19. "COVID coagulopathy"

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manifests mainly as a prothrombotic state affecting both large and small blood vessels, and presenting as arterial, venous, and microangiopathic thrombotic events with von Willebrand factor (VWF) and soluble thrombomodulin increased in hospitalized patients. The causes of coagulopathy are poorly understood. **Aim:** To investigate the relationship between von Willebrand factor (VWF) biomarkers, intravascular hemolysis, coagulation, and organ damage in COVID19 patients and to study their association with disease severity and mortality. **Methods:** 181 hospitalized adult COVID19 patients were randomly selected with a balanced distribution of survivors and non-survivors during the period of March 26th 2020 to May 5th 2020. The medical records and laboratory values were reviewed. Statistical analysis was performed using R studio V.3.6.2. **Results:** Patients who died (n=90) had significantly lower ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity, elevated lactate dehydrogenase levels, increased schistocyte/RBC fragment counts, and elevated VWF antigen and activity levels compared to patients discharged alive (n=91). In 31 patients, we measured several of these biomarkers on two or more occasions. The trending of ADAMTS13 activity illustrated that it is not steady throughout the hospitalization course. ADAMTS13 activity levels tended to improve and/or reach normal levels in patients that survived, yet ADAMTS13 activity levels worsened in most patients that died. Likewise, the VWF antigen and activity levels tended to decrease in patients that survived whereas tended to increase well above the normal range (2-3 folds) in patients that died. D-Dimer levels trended downwards in survivors, sometimes to levels less than 1 µg/ml, yet tended to increase in patients who died. Given the relationship between ADAMTS13 activity and mortality, we wanted to determine a cut-point of initial ADAMTS13 activity (within 72 hours from admission) to predict mortality. 102 patients in our cohort had an ADAMTS13 activity measurement within this timeframe. We determined that this optimal cut-point of initial ADAMTS13 activity was 43% using Youden's J statistic. Only 30% of patients who had an ADAMTS13 activity level of less than 43% on admission survived, yet 60% of patients survived who had an ADAMTS13 activity level of greater than 43% on admission. **Conclusions:** COVID-19 may present with low ADAMTS13 activity in a subset of hospitalized patients. Presence of schistocytes/RBC fragment and elevated D-dimer levels on admission may warrant a work-up for ADAMTS13 activity and VWF antigen and activity levels. These findings indicate the need for future investigation to study the relationship between endothelial and coagulation activation and the efficacy of treatments aimed at prevention and/or amelioration of microangiopathy in COVID-19.

Role of CD200 in the Detection of Nonhematologic Neoplasms by Routine Flow Cytometry Analysis

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Objectives: While flow cytometry is routinely used in the diagnostic work-up of hematolymphoid malignancies, its role in identifying non-hematolymphoid neoplasms is controversial. While a diagnosis of "non-hematolymphoid process" may be suggested by flow cytometry, typically, CD45-negative entities are not further characterized by their immunophenotypic profile. Some markers, such as CD56, have been well documented in non-hematolymphoid malignancies, such as high-grade neuroendocrine carcinomas (Stacchini, et al, 2018; Bryson et al, 2002). Other cell surface markers that are routinely studied with flow cytometry panels, such as CD200, are less well-described in the literature with regards to their presence/absence in non-hematolymphoid processes. We examined all flow cytometry cases from our institution over a 5-year period to identify trends in the immunophenotypes of non-hematolymphoid malignancies that were known to be CD45-negative and CD56-positive by flow cytometry.
Methods: We examined 3634 flow cytometry cases (2015-2020) and identified non-hematolymphoid cases based upon lack of CD45 expression. After excluding multiple myeloma cases from the CD45-negative entities, we were left with 19 CD45-negative cases. Chart review of these cases confirmed them as non-hematolymphoid by concurrent surgical pathology/cytopathology studies. Of these 19 cases, 2 were excluded because CD56 was not evaluated.
Results: Of the 17 CD45-negative/CD56-positive cases, 16 showed CD200 positivity (94%). Of these CD200-positive cases, 10 were ultimately diagnosed as small cell carcinoma (59%), 1 was diagnosed as Merkel cell carcinoma (6%), 1 was diagnosed as melanoma (6%), 1 was diagnosed as poorly-differentiated carcinoma (6%), 1 was diagnosed as Ewing-like sarcoma (6%), and 2 were unclassified further (12%). The single CD200-negative case was diagnosed as poorly-differentiated acinar cell carcinoma (6%). All cases of small cell carcinoma that were evaluated by flow cytometry showed expression of CD200 and CD56.
Conclusions: Our findings suggest that in CD45-negative/CD56-positive non-hematolymphoid malignancies, particularly small cell carcinoma, CD200 is frequently positive. CD200 was also found to be positive in rare cases of other non-hematolymphoid malignancies within the differential diagnosis of small round blue cell tumors. These findings indicate that CD200 may be a useful marker in suggesting the possibility of small cell carcinoma in non-hematolymphoid specimens that are evaluated by flow cytometry.

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