After receiving <30 mL of platelets, he developed back pain and headache and the transfusion was aborted. His wife informed the primary provider that her husband was IgA-deficient. When Transfusion Medicine was notified, a review of his EMR showed undetectable IgA (<6 mg/dL: reference: 46-236 mg/dL) and IgM (<25 mg/dL; reference 43-279 mg/dL) and mildly increased IgG (1877 mg/dL; reference 650-1643 mg/dL) from 2014. Additionally, a high-titer IgG anti-IgA (>1000 U/mL; reference <99 U/mL), had been reported. In lieu of these findings, we changed his transfusion requirements to issue only washed PRBCs and IgA-deficient platelets and plasma, but he has not required any blood products since the last reaction. While a definitive cause and effect has not been established, this case suggests that severe IgA- and IgM-deficiency with IgG anti-IgA may be associated with nonspecific symptoms even with the transfusion of small volumes (<75 mL). To our knowledge, similar reactions have not been previously reported.

WeakPrecipitin Ring from A Fecal Specimen with Markedly Elevated Alpha-1 Antitrypsin Level Measured by Radial Immunodiffusion

Yi Xiao, Edward Leung; Children’s Hospital of Los Angeles, Keck School of Medicine, University of Southern California

Radial immunodiffusion (RID) is a classic methodology for antigen quantification that relies on the development of a distinct precipitin ring from precipitated antigen-antibody complex. As the precipitin ring grows, the precipitate at the inner edge of the ring constantly dissolves due to excess antigen flooding from the point of application, while new precipitate forms at the leading edge of the ring. RID plates with anti-human alpha-1-antitrypsin are prepared in our lab to measure fecal alpha-1 antitrypsin (A1AT) to aid in the diagnosis and monitoring of Protein Losing Enteropathy (PLE). The procedure has routinely produced precipitin rings with small radii and distinct edges after incubating at room temperature for 48 hours. Unexpectedly, a fecal specimen from a patient produced an extremely weak and large precipitin ring that could have been easily overlooked. Dilution studies confirmed a highly elevated A1AT level of 67 mg/dL. The very weak and large precipitin ring was reproduced with a spiked specimen with similar A1AT concentration and kept expanding for several days until a distinct ring was formed eventually. Our data highlights a rare example of high-dose hook effect in RID and calls for meticulous attention and caution when reading and interpreting gel-based immunoassays with unexpected markedly elevated results to avoid additional confirmatory testing. In these cases, we recommend repeat testing with diluted specimens.

Patterns and Utility of Calprotectin in Patients with Microscopic Colitis

Ibrahim Abukhiran, Matthew Krasowski, Andrew Bellizzi, Sarag Boukhar, Anna Merrill; University Of Iowa Hospitals and Clinics

Context: Calprotectin is a cytoplasmic-protein that is released upon neutrophilic activation. Measuring fecal-calprotectin (FC) is used for monitoring inflammatory bowel disease activity and distinguishing it from irritable bowel syndrome. However, its utility in other types of colitis has not been well-investigated.

Design: Cases of collagenous-colitis (CC) and lymphocytic-colitis (LC) between 2015 and 2020 were retrieved from our institution surgical pathology database. Endoscopy and histopathologic examination findings were reviewed to confirm the diagnosis. 15 CC and 13 LC cases were included as FC was done at the time of initial diagnosis (before therapy). 62 cases of normal endoscopy and histopathologic examination were selected as a control group. One-way analysis of the variance (ANOVA) and receiver operating curve (ROC) analysis of FC were performed.

Results: Abnormally elevated FC (>50 ug/g) was identified in 77% and 64% of CC and LC cases, respectively. Only 1.6% of control cases had mildly elevated FC of 54 ug/g. The mean FC of CC and LC groups (246 and 214, respectively) were significantly higher than the control group (22.4); p < 0.05. LC and CC groups had no statistically significant difference in the mean FC (p = 0.8). The area under the curve was 0.93 with ROC analysis. At the suggested cut-off of 50 ug/g, the sensitivity was 78.6%, specificity was 98.4% with a likelihood ratio of 48.7.

Conclusions: Fecal calprotectin can be elevated in patients with lymphocytic or collagenous colitis, however with no statistically significant difference between the two types. Therefore, it has the potential to be used as a marker for screening, diagnosis, and monitoring response to therapy in patients with microscopic colitis.

VariantDirect: An extraction-free screening approach to detect circulating SARS-CoV-2 virus strains from pooled specimens

Abdulrahman Saadalla, Brooke Stroup, Bijal Parikh; Washington University in St. Louis

Coronavirus disease (COVID-19) caused by the SARS-CoV-2 virus has exposed clinical laboratories to unprecedented challenges. With surging case numbers, clinical laboratories were forced to acquiesce and integrate multiple testing platforms with varying workflows and analytical sensitivities in order to meet testing volumes. Now a new challenge has emerged with the evolution of viral
variants, both globally and locally, raising concerns for uncontrolled spread, increased disease severity, and weakened responses to vaccinations. Preliminary data suggests that these variants may be associated with higher viral titers and prolonged infections. While primarily leveraged for epidemiologic surveillance, the clinical utility of variant detection may quickly become paramount. Furthermore, laboratories must remain vigilant and nimble enough to pivot should variant identification play a role in the patient care. To prepare for the validation of clinical assays that identify important viral variants, we designed a novel method, termed VariantDirect, to screen SARS-CoV-2 positive samples for the presence of variants, focusing initially on the increasingly prevalent UK and South African (SA) variants. The detection strategy is based on primers designed to specifically target the viral receptor-binding domain mutation, N501Y, shared by the UK and SA strains. Screening for variants will be limited to nasopharyngeal swab samples of high viral titers (Ct values <25 by RT-qPCR assay, Roche Diagnostics). Pools of 9 different samples, 50 µl each, are mixed and stored at -80°C along with aliquots of the 9 original samples. These pools will then be tested, and if positive for the N501Y variant, the pooled 9 samples will be thawed and tested separately to identify the affected specimen. Most of these specimens are also being independently sequenced via a comprehensive but more resource-intensive NGS approach. Advantages of our pooled workflow are primarily in time and cost, with the capacity of screening up to 837 specimens on a single run. In addition, our collection strategy establishes a “time capsule” to document the evolution of viral strains within our geographical region. Finally, these studies serve to optimize technical parameters for the development of clinical assays. A validated nucleic acid (NA) extraction-free RT-qPCR method will be utilized for this assay. Our internal validation data showed comparable analytical sensitivities to NA extraction-based methods. Pooled samples in transport medium are diluted in normal saline at a ratio of 1:1, and then heat-inactivated in the presence of proteinase-K and ultimately analyzed on the Applied Biosystems™ 7500 Fast Dx instrument. As new variants of interest emerge, primers and probes can be quickly redesigned and validated on clinical samples within our NGS-confirmed “time capsule”. This study will provide important information needed for current or future genomic and epidemiologic studies.

Combined utility of Glucose and HbA1C testing for screening diabetes mellitus

Vishnu Samara, Kathleen Kelly, Lee Hilborne; University of California Los Angeles

Screening for diabetes mellitus is accomplished by measuring fasting blood glucose or HbA1C. The American Diabetes Association (ADA) guidelines recommend HbA1c for screening patients for diabetes or pre-diabetes, the US Preventative Services Task Force (USPSTF) includes HbA1c only for monitoring and either glucose or HbA1c can be used for screening. This project sought to provide clinical laboratory evidence to support HbA1c as a diabetes screening test. De-identified electronic health record (EHR) patient data from individuals visiting a large medical center and its affiliated clinics that were tested for blood glucose (either alone, basic metabolic profile or comprehensive metabolic profile) and HbA1c, ordered together on the same date of service were collected. 333,360 combined glucose and HbA1c requests were received in 2020. For further analysis, we included patients only with ICD-10 routine visit code Z00.00, excluding known diabetics, patients with elevated blood glucose and HbA1c below 5.7% because this combination may indicate a non-fasting or inadequate fasting state. From the patients with diagnosis code Z00.00 and glucose within the reference interval, 73% had HbA1c levels greater than 5.7%. Among them, 65% are of pre-diabetes [HbA1c between 5.7 and 6.4%] and 35% with HbA1c over 6.5%. Medical record review of patient charts with HbA1c over 6.5% suggested a diagnosis of diabetes and were prescribed hypoglycemic medications. Elevated glucose and HbA1c complement each other in the initial diagnosis for diabetes and pre-diabetes; where as HbA1c alone is a good indicator in screening diabetes and pre-diabetes individuals that were previously not diagnosed with diabetes. We are currently collecting 2019 data to examine the differences and adjust for the sample volume due to effect of COVID-19 pandemic on patient visits in the early 2020. We are also evaluating if other variables such as insulin levels, insulin resistance status, and their correlation with HbA1c as a screening measure.