Presenter's Name: Aziz, Sarah

Additional Author(s): Zeman-Pocrnich CE

Abstract Title: TLE1 expression in NUT carcinoma: a potential diagnostic pitfall

Abstract:

Introduction: NUT carcinoma is a rare, aggressive malignancy defined by a rearrangement affecting the nuclear protein in testis (NUT or NUTM1) gene. This small round blue cell tumour typically exhibits focal abrupt keratinization and immunohistochemical positivity for cytokeratin and squamous markers. Reports of positivity for other markers that may obscure the diagnosis are increasing. We describe a case of NUT carcinoma that initially mimicked an Ewing family sarcoma or monophasic synovial sarcoma due to strong CD99 and TLE1 staining.

Case: A man in his 40s presented with chest pain, dyspnea and upper extremity swelling due to a large mediastinal mass. Core biopsy showed a monomorphic small round blue cell tumour with abundant apoptosis and necrosis, and immunohistochemical positivity for cytokeratin (focal, weak), membranous CD99 (diffuse, strong) and TLE1 (patchy, strong). There was no evidence of squamous differentiation by morphology or immunohistochemistry. Initially, top differential diagnoses included an Ewing family sarcoma or monophasic synovial sarcoma; however, molecular testing confirmed a BRD4-NUTM1 fusion, diagnostic of NUT carcinoma. The patient initially responded to palliative radiotherapy, but died approximately one month after completion of radiotherapy.

Discussion: Positive TLE1 staining can occur in NUT carcinoma and can represent a diagnostic pitfall, particularly when other features typical of NUT carcinoma are absent. Referral to a centre that offers immunohistochemical, cytogenetic or molecular testing to detect the NUT translocation should be considered in select cases of small round blue cell tumours.

POSTER PRESENTATIONS 2
2A: TEST UTILIZATION, OPTIMIZATION & QA

Presenter's Name: Gomes, Janice

Additional Author(s): Janssen B, Penny J, Hur L, Pasternak S, and McIntyre CW

Abstract Title: Development of an Extracellular Vesicle-based biomarker of hemodialysis induced vascular injury

Abstract:

Introduction: Hemodialysis (HD) is a treatment for those with kidney failure. HD unfortunately produces negative side effects such as hemodynamic instability and microvascular dysfunction, which can lead to an increased risk of cardiovascular disease, stroke, and death. HD associated vascular damage and organ injury has been observed through imaging techniques such as echocardiography, computed tomography, magnetic resonance imaging, and positron emission tomography. These methods are time consuming and expensive, which is why blood-based biomarkers are needed. Current literature suggests that biomarkers of vascular injury are endothelial and platelet derived Extracellular Vesicles (EVs). However, the parameters that impact EV levels, size distribution, and their potential for clinical use among HD patients are unknown. Therefore, we aimed to determine whether endothelial and platelet derived EVs are appropriate candidate biomarkers of HD associated vascular injury.

Methods: To determine parameters of EV release, Human Umbilical Vein Endothelial Cells (HUVEC) were exposed to stressors (Lipopolysaccharide (LPS), HD serum, and healthy control serum), and cell culture media was analyzed for EV levels and size distribution. To understand the effects of HD we developed an in vivo rat model of HD. We exposed rats to a 2-Hour HD session, collected blood samples at various time-points for EV analysis, and simultaneously measured blood pressure and microcirculation through intravital microscopy. Additionally, we collected pre- and post- HD blood samples from HD patients for EV analysis. We also recorded ultrafiltration rate, changes in blood pressure, and cardiac stunning.

Results: When HUVEC were exposed to LPS, there was an increase in small sized endothelial EVs (<500nm) compared to the control. Moreover, by utilizing the rat model of HD, we observed a significant decrease in microcirculation, while observing an increase in endothelial and platelet EV levels over the course of HD. We also observed a significant increase in endothelial EV levels from pre- to post- HD among patients. Pre-HD endothelial EV levels correlated positively with ultrafiltration rate and cardiac stunning, whereas Pre-HD platelet EV levels correlated with a decrease in systolic blood pressure through HD.

Discussion: Our overall findings suggest that endothelial and platelet EV levels and their size distribution may be indicative of HD-induced vascular injury.

Presenter's Name: Jasani, Arish

Additional Author(s): Rutledge A, Stevic I, Bhayana V

Abstract Title: Drug interference studies on clinical chemistry tests at London Health Sciences Centre

Abstract:

Introduction: Clinical chemistry laboratory tests work on many different principles, including spectrophotometric and colorimetric designs. During analysis, there is potential for assay interference if the patient's biological specimen contains anything endogenous or exogenous (such as a drug) that can absorb at the assay wavelengths. These drugs tend to absorb light in a similar spectrum to the absorption spectrum of these laboratory tests and may lead to erroneous diagnosis of diseases. Metronidazole and methylene blue absorbing wavelengths around 340 nm and 550 – 700 nm respectively have been previously seen to interfere with some chemical tests from different manufacturers using a similar absorption spectrum.

Methods: For each potential interfering drug, assays that used a wavelength around the absorbance peak of the drug were selected to test for interference. A pool of biological specimens from different patients containing the analyte of interest at desired (clinically relevant) concentration ranges is made to be tested by a clinical chemistry test. The pooled analyte sample is aliquoted and spiked with a higher and a lower concentration of the selected potentially interfering drug separately, while same volume of water is used to spike the control aliquots. After performing these tests, the difference in concentration between experimental and control are reviewed against the total allowable error to identify if interference has occurred.

Results: There was no interference observed with Roche chemical assays when metronidazole was present in the samples. With methylene blue, we observed interference with the urine amphetamine screen and urinalysis tests. Methylene blue did not interfere with any of the plasma/serum assays tested, except for the lipemic index.

Discussion: This study provides reassurance that metronidazole is not interfering with the chemistry assays used at our site. For methylene blue, now that affected tests have been identified, we will investigate measures to limit release of erroneous results in patients treated with this medication. Our findings will also be beneficial for other laboratories using chemical tests from the same manufacturer.

POSTER PRESENTATIONS 2
2A: TEST UTILIZATION, OPTIMIZATION & QA

Presenter's Name: Lad, Mrinal

Additional Author(s): Hsia C, Chin-Yee B, Hedley B, Chin-Yee I

Abstract Title: A survey of current clinical practice in the management of Monoclonal B-cell Lymphocytosis (MBL): Are we over-investigating?

Abstract:

Introduction: Monoclonal B-cell lymphocytosis (MBL) is an indolent, hematologic condition presumed to precede all cases of chronic lymphocytic leukemia (CLL). MBL is defined by an excessive monoclonal B-cell population in the blood, but most MBL patients remain asymptomatic and otherwise healthy. Although MBL is common in older adults, only the high-count MBL subtype is of clinical significance because it can progress to CLL at a rate of 1-2% annually. Recently, studies have provided favourable evidence towards using the CLL-International Prognostic Index (CLL-IPI) to risk-stratify all patients with MBL. However, this risk-stratification process requires costly molecular testing to conduct such as flow cytometry so it is important to consider when the appropriate time is to administer testing and what is the right test to administer. The purpose of the current study is to determine if current clinical practices are over-investigating this indolent condition.

Methods: A retrospective chart review will be conducted using health records from the Hematology Clinic at London Health Sciences Centre. Clinical variables and laboratory parameters will be recorded and compared between CLL and MBL patients to understand patient characteristics of those diagnosed with MBL and to compare rates of testing between the two patient populations.

Results: It is expected that CLL-IPI scores will change clinical management between MBL patients as those placed in a higher risk group will receive significantly more testing compared to patients placed in a lower risk group. Similarly, it is expected MBL patients will have similar rates of testing to early-stage CLL patients.

Discussion: The results of this novel descriptive study will provide an accurate description of the standard of care for patients with MBL and may contribute to reconsiderations in testing procedures. Consequently, it will help improve healthcare quality and resource utilization for patients with MBL.

Presenter's Name: Li, Shao Shi

Additional Author(s): Cecchini MJ, Zeman-Pocrnich CE

Abstract Title: Adequacy of cytopathology samples for lung panel molecular testing at LHSC

Abstract:

Background: Minimally invasive techniques are increasingly used for tissue acquisition from patients with suspected lung cancer, and it is essential that the small biopsy and cytopathology (CP) samples derived from these procedures be sufficient for both diagnosis and molecular testing (MT). In this study, we set out to determine the adequacy of CP samples for Lung Panel MT at our centre and reasons that CP samples fail MT. This information will allow us to optimize CP samples for MT.

Method: For a 1-year period (mid 2019-mid 2020), the total number of surgical pathology (SP) and CP blocks submitted for Lung Panel MT was determined. SP and CP blocks with insufficient amplifiable DNA for analysis were identified, and insufficient rates were calculated. Slides from insufficient CP blocks were reviewed in order to determine why the sample failed MT.

Results: Over the study period, 527 blocks underwent Lung Panel MT including 401 SP blocks and 126 CP blocks. There were 11 insufficient SP blocks (2.7%) and 7 insufficient CP blocks (5.5%). The 7 insufficient CP blocks originated from 6 separate CP cases (5 EBUS/EUS-FNAs of carcinoma in mediastinal/hilar lymph nodes and 1 FNA of carcinoma at a soft tissue site). Each of the 7 failed CP blocks had immunoperoxidase (IP) slides ordered prior to the molecular cuts (average of 5 IP slides/block). For the 7 failed CP blocks, the number of tumour cells on the last level before the molecular cuts, as determined using QuPath, ranged from 1,198 to 18,442 cells (average 6,139 cells). It is also interesting that there was 1 insufficient CP case where an alternative cell block could have been submitted for repeat MT had the failed MT result been flagged.

Discussion: The MT insufficient rate at our centre for CP samples is 5.5%, as compared to 2.7% for SP samples. Based on tumour cellularity on the last slide prior to the molecular cuts, we would have expected many of the failed CP blocks to have been sufficient for MT. Potential reasons for the unexpected failure include i) excess refacing of the block at microtomy and ii) suboptimal DNA extraction, perhaps especially problematic in cases lacking uniform distribution of tumour cells within the block. Improved communication between the pathologist selecting the area for extraction and the technologist performing the extraction and/or consideration of alternative extraction techniques may improve the success of MT in CP samples.

POSTER PRESENTATIONS 2
2A: TEST UTILIZATION, OPTIMIZATION & QA

Presenter's Name: Santaguida, Vincent

Additional Author(s): Elzagallaai A, Rieder M

Abstract Title: Optimization of Cortisol Extraction from Human Hair

Abstract:

Cortisol is a glucocorticoid hormone that plays an important role in both normal physiology and numerous diseases. Cortisol has traditionally been measured using blood, urine and saliva samples, however due to the daily fluctuations in cortisol levels, these measurements tend to be inconsistent depending on when the samples are obtained. As such, the measurement of cortisol from human hair has been used clinically due to its ability to obtain consistent and retrospective measurements. However, current methods for extracting cortisol from hair only extract about 50% of the total cortisol in the hair, and these partial extractions may reduce the accuracy of this method. As such, this study aims to optimize the extraction procedure for hair cortisol measurement and analyze the effects of various changes to the extraction process. First, we will compare the efficiency of milling and mincing the hair. Then, we will compare the effect of doubling the time of the extraction, repeating the extraction twice, and a 4-step extraction using methanol and acetone, to the standard procedure. We do not expect there to be a difference in the amount of cortisol extracted when milling and mincing the hair. However, we expect longer and double extractions to extract more cortisol than the standard procedure, and the 4-step methanol-acetone extraction to extract the most cortisol. The ability to extract all, or close to all of the cortisol in hair would allow for the generation of more precise values and standard reference ranges for hair cortisol. Ultimately, this could be beneficial for the diagnosis and treatment of many cortisol related diseases.

Presenter's Name: Smuland, Alexandria

Additional Author(s): Cecchini MJ

Abstract Title: Specificity of Lymph Nodes Identified Macroscopically from Colorectal Resection Specimens.

Abstract:

Introduction: For resected colorectal specimens, the pericolic soft tissue is dissected in search for lymph nodes (LNs). LNs are encapsulated and highly organized collections of immune cells and are the first site of cancer metastasis. Involvement of the lymph nodes is a critical element of cancer staging and guides further treatment decisions. LNs vary in size and macroscopically identifying all these nodes is a challenging time-consuming process. We hypothesize that there is a significant quantity of non-lymph node (N-LN) tissue submitted during macroscopic examination as potential LN tissue. Understanding and improving this specificity could result in efficiency gains and cost savings.

Methods: Cases of colon cancer without LN metastasis were identified (n=50) and the LN slides were reviewed. The number, size and presence of LN tissue was quantified for each of the tissue fragments submitted for microscopic examination. Smaller LNs are submitted together as intact tissue fragments while larger LNs are serially sectioned and submitted in their own blocks. These two types of LNs were considered and analyzed separately. A cost analysis was performed based on the minimum number of slides that could have been utilized if all fragments contained LN based on standard operating procedures for lymph node submission.

Results: The specificity of LN submission where LNs were submitted as one per cassette was found to be 92.86% whereas the specificity of LN submission for intact LNs was found to be 56.63%. The average size of the submitted LNs was 0.39 cm with a range of 0.1 to 1.8 cm. While the average size of the fragments without LN tissue was 0.65 cm with a range of 0.2 to 1.7 cm. Based on the 50 cases reviewed, we identified that on average an extra 2.82 blocks of tissue were submitted per case at an estimated cost of $56.40 per case.

Discussion: Improving the specificity in LN submission could help improve the efficiency and produce cost savings for the pathology lab. However, given the importance of the LNs for staging and treatment decisions it would not be acceptable to sacrifice the sensitivity to improve specificity. Targeted training and feedback of staff may improve the specificity. Novel technologies could also provide useful adjuncts to the traditional macroscopic assessment and hold the promise to improve sensitivity and specificities.

POSTER PRESENTATIONS 2
2A: TEST UTILIZATION, OPTIMIZATION & QA

Presenter's Name: Suthakaran, Abitha

Additional Author(s): Chin-Yee B, Chin-Yee I, Hsia C

Abstract Title: T Cell Clonality - Are we identifying pathology or incidental clones? A quality improvement project

Abstract:

T cell clonality testing is used to identify clonal populations of T cells in patients by looking at gene rearrangements in the T cell receptor (TCR). While T cell clonality is a clinical feature of many lymphoproliferative disorders such as cutaneous T cell lymphoma (CTCL) and T cell large granular lymphocytic leukemia (T-LGL), clonal T cell populations have also been identified in healthy elderly individuals. Therefore, clonality does not imply malignancy or pathology. This raises the question of whether T cell clonality assessment is being overutilized to investigate incidental clonal populations, and whether these incidental clonal populations are being misattributed to disease. The goal of this project is to survey the use of T cell clonality testing to figure out the impact on patient management and to improve test utilization. We hypothesize that a large population of patients undergoing T cell clonality assessment are not diagnosed with a lymphoproliferative disorder, as well as that a significant portion of T cell clones identified through testing are incidental or of undetermined significance. A retrospective descriptive study will be conducted at London Health Sciences Centre using records of patients who underwent T cell clonality testing between January 1st, 2011 and September 30th, 2021 and were over the age of 18 at the time of testing. Descriptive statistics will be used to analyze data collected on patients' demographics, medical history, laboratory tests (e.g. hematology), and post-test management to characterize the patient population for T cell clonality testing and devise an algorithm to guide physicians on when to order this test. We expect to observe significant clinical differences between patients with incidental clones and those with clones associated with lymphoproliferative disease. We also expect to determine that a large portion of tested patients have incidental clonal populations. These findings will help improve clinic resource management as well as patient outcomes by preventing unnecessary testing.

Presenter's Name: Tran, Hanh

Additional Author(s): Schofield S, AlMutawa F, Delport J, Elsayed S, Fuller J, Payne M, Cabrera A

Abstract Title: Validation of an 8-plex real-time reverse transcription - polymerase chain reaction (RT-PCR) assay for the detection of multiple respiratory viruses

Abstract:

Introduction: Diagnostic testing for seasonal respiratory viruses plays an important role in patient care. To improve the efficiency of the clinical workflow in our laboratory, we developed and validated a fully automated multiplex assay to identify common viral pathogens associated with seasonal respiratory infection. A two-tube assay was designed for the detection of 1) SARS-CoV-2, Influenza A, Influenza B, Respiratory Syncytial Virus (RSV), and, 2) Parainfluenza 1-4 (PIV 1-4), Metapneumovirus (hMPV), Adenovirus (AdV) and Picornavirus (Rhino/Enterovirus).

Methods: Negative specimens obtained from patients included nasopharyngeal swabs in transport media and bronchoalveolar lavages were spiked with known positive representative samples and used in our validation study. Samples were extracted using the Microlab STARlet IVD platform (Hamilton Company, Reno, Nevada, USA). Real-time reverse transcription-polymerase chain reaction (RT-PCR) was performed using the LightCycler (LC) Multiplex RNA Virus Master Mix (Roche Diagnostics, Indianapolis, Indiana, USA) on a Roche LC480 II (Roche Diagnostics). An internal control (RNAseP) was included in tube 1. Winterplex Modular kits (TIB MOLBIOL, Berlin, Germany) for Sarbecovirus E-gene, Influenza A, Influenza B and RSV were multiplexed with RNAseP primers and probe (IDT Integrated DNA Technologies, Coralville, Iowa, USA) in tube 1. Tube 2 combined separate LightMix Modular kits of PIV 1-4, hMPV, AdV and Picornavirus (TIB MOLBIOL).

Results: The accuracy was 99.77% for Influenza A, Influenza B and RSV, and 99.31% for SARS-CoV-2. The accuracies for tube 2 for PIV 1-4, hMPV, AdV and Picornavirus were 96.79%, 99.46%, 100% and 97.87%, respectively. No cross-reactivity was observed. Precision was calculated from triplicates over five days. All targets had a coefficient of variation (CV) < 5%.

Discussion: The multiplex assay described here performed well, allowing for simultaneous detection of multiple seasonal respiratory viruses in a single RT-PCR assay. Implementation of this assay into the clinical workflow fostered expedited testing and reporting of seasonal respiratory viruses. Automation of the extraction and liquid handling components of the assay also decreased hands-on time.