HLA-DR, increased CD33, increased CD123, variably decreased CD38, variably decreased CD13 and variable CD117 as the most characteristic pattern. The disease occasionally presented as expanded immature monocytes with or without increased CD56. The LAI associated with NPM1+ AML was relatively stable post therapy and in relapse, unless significant genetic alteration occurred. 12 patients had MRD at a level less than 1%. Among them, 5 had MRD at the end of induction and 1 relapsed. In comparison, 7 patients had MRD post chemotherapy before or after allogeneic bone marrow transplant and all 7 patients relapsed.

Conclusions: Flow cytometry immunophenotying offers high sensitivity to detect MRD in NPM1+ AML. The presence of LAI defined MRD post therapy predicts disease relapse.

Infectious Disease Pathology

1532 Prevalence of Adenovirus Colitis in Stem Cell Transplant Recipients

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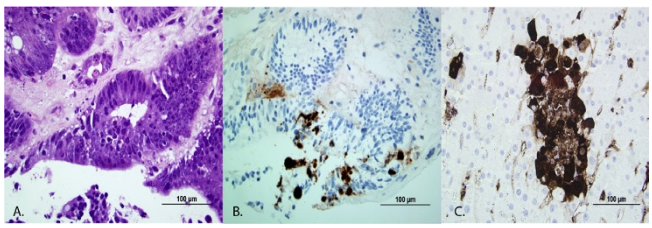
Background: There are numerous morphologic similarities among viral infection (including cytomegalovirus (CMV) and adenovirus) in gastrointestinal (GI) biopsies from patients who have undergone stem cell transplantation (SCT) which include graft-versus-host disease (GVHD) and drug toxicity. It is very challenging and may pose a diagnostic dilemma to distinguish these entities in a pathologic diagnosis. The common morphologic features of these diseases are crypt apoptosis with minimal to absent periglandular inflammatory infiltrate. Routine CMV immunostaining in suspicious GI biopsies with apoptotic crypts is commonplace, but not for adenovirus. The prevalence of adenovirus infection in SCT recipients with an early GVHD-like histologic picture is not well studied, and the significance of recognizing and diagnosing this infection is tantamount for proper treatment.

Design: A total of 37 patients status post stem cell transplantation were collected for this study. Among all endoscopic colon biopsies from these patients, a prior diagnosis of early GVHD or suggesting GVHD were included for further assaying with adenovirus and CMV. Immunohistochemistry (IHC) for adenovirus and CMV were performed for all cases with proper positive and negative controls.

Results: In this cohort, all patients were on immunosuppression at the time of biopsy and clinically presented with diarrhea. Morphologically all cases displayed a minimal chronic inflammatory infiltrate with crypt apoptosis and no CMV was identified immunohistochemically. One of the biopsies showed clusters of cryptal epithelial cell necrosis/apoptosis with sandy/smudgy nuclei (Fig.1a), and positivity for adenovirus was determined immunohistochemically (Fig. 1b). Further contrast-enhanced CT imaging showed a normal-sized liver with numerous ill-defined foci; liver biopsies were performed and adenovirus hepatitis was identified (Fig.1c).

Conclusions: Our study indicates that adenovirus colitis is an uncommon phenomenon among patients status post SCT who present with a GVHD-like histologic profile. Although currently adenovirus IHC is not routinely performed for biopsies of SCT patients, morphology and IHC could serve as a practical approach for patients with suspicion to have adenovirus infection.



1533 Staph Pseudintermedius: An Emerging Pathogen

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Background: *Staphylococcus pseudintermedius* is a normal inhabitant of dog and cat skin and mucosa that was first differentiated from *S. intermedius* as a novel species in 2005. It rarely has been described in humans, but the true incidence of this possibly emerging pathogen is not known because it has been misidentified in the past as *S. aureus* or *S. intermedius*. Both *S. pseudintermedius* and *S. intermedius* are Gram-positive cocci. Both grow aerobically and are catalase and coagulase positive. Newer, more precise diagnostic tools such as MALDI-TOF mass spectrometry, however, have led to the identification of more cases of *S. pseudintermedius*.

Design: The authors retrospectively identified patients during a 3.5-year period at a tertiary care center in the U.S. with cultures that identified *S. pseudintermedius* and *S. intermedius*. Data were abstracted from the patients' electronic charts and their clinical presentation and predisposing conditions were examined.

Results: We identified 15 cases of *S. intermedius/pseudintermedius* from 1/1/2012 to 6/31/2015. 13/15 (87%) were identified using MALDI, the others with Microscan. 8/15 (53%) were identified as *S. intermedius* and 7 as *S. pseudintermedius*. In 12/15 (80%), the *S. intermedius/pseudintermedius* species identified was concomitant with other bacterial organisms. 8/12 patients identified (53%) had significant co-morbidities including rectal cancer, colon cancer, lung carcinoma, Crohn's disease, coronary artery disease with coronary artery bypass graft, diabetes mellitus and advanced COPD with lung transplant. 4 patients (27%) had no co-morbidities. In 10/15 cases (67%), the pathogen was identified in wound infections. 3 cases (20%) resulted from dog bites.

Conclusions: *S. pseudintermedius* is an opportunistic pathogen and a leading cause of skin and ear infections, infections of other body tissues and cavities and post-operative wound infections in dogs and cats. The 15 cases of *S. pseudintermedius* and *S. intermedius* we identified raise the question of whether cases of the two pathogens are on the rise or if the organisms are being identified because of more precise diagnostic capability. The occurrence of *S. pseudintermedius* in human infections is probably underestimated because commercial systems for fast and correct identification of this pathogen are not available. It also is a newly described species that is not included in databases of most systems, and because it is coagulase-positive is likely identified as *S. aureus* in many clinical laboratories. The increasing use of MALDI identification technology will assist laboratories in the correct identification of this emerging pathogen.

1534 Morphoproteomics to Define Targets for Host-Directed Therapy in Tuberculosis

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Background: Tuberculosis (TB) is an infectious disease that progressively modifies lung tissue due to host immune responses against pathogenic antigens. The search for biomarkers of disease is hypothesized to be necessary towards development of novel and effective therapy that will not be hampered by antibiotic-resistance. However, lack of lung tissue from untreated and/or poorly treated TB individuals has limited findings. Most, if not all, biomarker analysis is conducted on PMBCs or BAL isolated cells, which only allows profiling of peripheral host responses. Using morphoproteomics, developed to guide cancer host-directed therapy, we demonstrate for the first time expression patterns of foamy alveolar macrophages at the site of TB disease.

Design: Lung sections collected from autopsy of individuals with untreated and/or poorly treated TB disease. FFPE sections were stained with H&E, AFB, and anti-TB antigen stain. Immunohistochemical staining was completed using anti-human PD-1, PD-L1, COX-2, p-mTOR (Ser 2448), p-AKT (Ser 473), and IGF-1R.

Results: Pathology analysis demonstrates the presence of foamy macrophages in alveolar spaces. Presence of TB pathogen is significantly less compared to the presence of TB antigens in these activated foamy macrophages, suggesting that spread of TB antigens without high pathogenic load may be the driving force of foamy macrophage formation.

Foamy macrophages are heavily positive for expression of activated, phosphorylated (p)- mTOR. Additionally, pmTOR is also positive in the alveolar walls, but to a lesser intensity. There was minimal presence of p-AKT in the foamy macrophages. This lack of p-AKT suggests that during MTB infection foamy macrophages are overexpressing mTORC1 and little activation of mTORC2.

Foamy macrophages varied in COX-2 intensity. In the MTB infected lung microenvironment, PD-L1 is highly expressed in foamy macrophages, surrounded by PD-1 expressing lymphocytes in the interstitial tissues. This suggests that foamy macrophages in the MTB infected lung sets up an environment favoring T effector cell suppression, preventing control of MTB infection.

Conclusions: These preliminary results demonstrate a unique pattern of biomarker expression in alveolar macrophages at the site of TB disease and identify effective targets for host-directed therapy. We hypothesize that a combination of metformin (mTOR inhibitor) and celecoxib (COX-2 inhibitor) could be effective as an adjunct to current TB antibiotic therapy regimen.

1535 Validity of Minimally Invasive Autopsy (MIA) in Assessing Cause of Death in Adults from Developing Countries

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Background: There is an urgent need to accurately estimate the cause of death (CoD) in low-income regions. Current methods (complete diagnostic autopsy [CDA], verbal autopsy and clinical records) are either inaccurate or poorly acceptable and/or feasible. We aimed to analyze the validity of a standardized minimally invasive autopsy (MIA) in the evaluation of the cause of death in a series of adults from Maputo, Mozambique.

Design: Coupled MIA and CDA were performed to a series of 112 in adults who died at the Maputo Central Hospital. The MIA procedure involves the collection of blood and cerebrospinal fluid (CSF) and puncture of liver, lungs, heart, spleen, kidneys, bone marrow and brain using biopsy needles. The histological and microbiological examination of the MIA was done blindly, without any knowledge of the clinical data or the results of the CDA. The routine microbiological evaluation included conventional cultures, analysis for HIV, malaria and tuberculosis, and specific PCR analyses guided by histology results. The putative CoD obtained in the MIA was compared with the results obtained in the CDA.

Results: Final CoDs for the 112 deceased adults, as determined in the CDA, included infectious diseases (77; 69%), malignant neoplasms (16; 14%), and cardiovascular diseases or non-infectious respiratory (16; 14%). CDAs were non-conclusive in 3 cases (3%). Complete agreement between MIA and CDA was identified in 99 cases (88.4%). The agreement was higher for infectious diseases (73/77; 94.8%) than for malignant neoplasms (13/16; 81.3%) or for cardiovascular or non-infectious respiratory diseases (10/16; 62.5%). Interestingly, the specific agent was identified in the MIA in 58/77 (75.3%) patients dying of infectious diseases.

Conclusions: A simplified MIA technique allows obtaining adequate material from body fluids and major organs leading to accurate diagnoses. This procedure could improve the determination of CoD in developing countries.

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1536 Investigation of Potential Misdiagnosis of Corynebacteria as Mycobacteria Based on Acid Fast Stains in Surgical Pathology Specimens

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Background: In current surgical pathology practice, acid-fast stains including Ziehl-Neelson and Fite stains are commonly employed to identify *Mycobacteria* sp. Positive staining by these methods is due to mycolic acids present within mycobacterial cell walls. Other non-*Mycobacteria*, such as *Corynebacteria* species, also contain mycolic acids and are known in clinical microbiology to occasionally stain partially acid-fast. However, their staining characteristics in histologic sections are not well characterized in surgical pathology literature. *Corynebacteria* are frequently present as normal human flora and theoretically could stain acid-fast positive, potentially leading to their misidentification as *Mycobacteria*.

Design: Cultures of *Corynebacterium* species known to contain mycolic acids (*C. striatum* and *C. matruchotii*) and known to not contain mycolic acids (*C. amycolatum* and *C. kroppenstedii*) were incubated overnight on tryptic soy agar with 5% sheep blood (SBA) and on egg yolk agar (EYA). Pure colony material from each media type and for each species was emulsified into sterile water on a sterile glass microscope slide and heat fixed for 10 minutes. All slides were stained with standard staining protocols used in clinical microbiology (modified-Kinyoun method) and surgical pathology (Fite-Faraco method and Ziehl-Neelsen method). The stained slides were reviewed in a blinded fashion.

Results: All four species of *Corynebacterium* demonstrated a similar pattern of staining by the modified-Kinyoun method for both SBA- and EYA-grown cultures; there were uniformly distributed, focal areas of acid-fast positive bacilli in a background of predominantly acid-fast negative bacilli. None of the four species were acid-fast positive by the Fite-Faraco or Ziehl-Neelsen method for both SBA- and EYA-grown cultures. Positive and negative controls demonstrated expected staining patterns.

Conclusions: Despite the presence of mycolic acids in the cell walls of *Corynebacteria*, they reassuringly do not retain carbol fuchsin upon decolorization when performing Fite-Faraco or Ziehl-Neelsen methods of acid-fast staining. Nevertheless, the surgical pathologist should be made aware of this theoretical possibility, particularly when a diagnosis of *Mycobacteria* does not match the clinical picture.

1537 Human Parechovirus and Enterovirus Initiate Divergent Innate Immune Responses in the CNS: Pathogenic and Diagnostic Implications

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Background: The picornaviruses, enterovirus (EV) and human parechovirus (HPeV), cause a wide range of diseases including CNS infections, which can be severe and potentially fatal. EV causes most cases of pediatric meningoencephalitis worldwide, and HPeV type 3 is the most common cause of viral meningoencephalitis in infants younger than three months of age. The typical, early response to picornavirus infections involves the innate immune system and the release of cytokines, secreted proteins which coordinate immune system functions. In CNS infections, cytokines appear almost immediately in the CSF compartment. A robust antiviral state requires release of the cytokines essential to host defenses and the priming of adaptive immunity. We examined CSF of EV and HPeV meningitis cases for cytokines involved in the antiviral response in order to understand the innate immune activation of these infections.

Design: CSF samples from patients with HPeV and EV meningitis were analyzed for cytokines. Cytokine levels were determined in CSF samples from 14 HPeV cases (mean age 1.38 years), 13 EV cases (mean age 4.12 years), and 11 controls (mean age 3.04 years). A multiplex sandwich enzyme-linked immunosorbent assay (ELISA) was used to determine levels of IFNγ, IL-1a, IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70 and TNFα in CSF samples.

Results: CSF cytokine levels in EV meningitis patients were significantly elevated compared to control samples for IL-2 and, IL-6 ($p<0.05$). In contrast, CSF cytokine levels of HPeV meningitis were not different from controls and were significantly lower than EV meningitis for IL-2, IL-4, IL-6, IL-8, IL-12p70 and TNFα. Additionally, TNFα levels were significantly lower in HPeV meningitis when compared to controls ($p<0.05$).

Conclusions: The distinct patterns of cytokine expression in these two viral infections reflect their different pathogenic mechanisms: neuronal infection by EV and smooth muscle cell infection by HPeV. The inappropriately low levels of the cytokines involved in viral activation of innate immune defenses in the HPeV meningitis cases suggest that HPeV evades innate immune detection, leading to a deficient antiviral state. The dramatically divergent cytokine patterns of these two viruses not only parallel disease pathophysiology but will aid in rapid diagnosis of neonatal meningitis cases.

1538 Yersinia Pestis Osteomyelitis: An Underreported Complication of Plague

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Background: Yersinia pestis (Y. pestis) has caused high mortality epidemics in human history. Three main forms of plague - bubonic, septicemic and pneumonic - affect humans. The Gram-negative obligate intracellular bacterium is blood born and affects multiple organs. Surprisingly, osteomyelitis is rarely described. We investigate the prevalence of bone involvement among Y. pestis cases submitted to the Centers for Disease Control and Prevention's (CDC) Infectious Diseases Pathology Branch.

Design: Following confirmation of Y. pestis osteomyelitis by immunohistochemistry (IHC) in an index case, the case records of the CDC were searched to identify additional cases.

Results: Of 12 total human cases of plague referred to the CDC from 1998 – 2015, three definite (3/12, 25%) and one suspected (1/12, 8%) case of Y. pestis osteomyelitis were identified. Patient age ranged from 13-60 years old and multiple bones were affected.

Age	Gender	Origin	Autopsy/ Surgical	Submitted Tissues	Tissues Positive by IHC
13	M	Ecuador	Autopsy	5th phalanx	5th Phalanx, papillary and reticular dermis
14	M	CA, USA	Surgical	L tibia, R hip synovial tissue	L tibia
53	M	NYC, USA	Surgical	L and R foot, viable and non-viable debrided tissues	L and R foot, non-viable tissues
60	M	CA, USA	Autopsy	Bone marrow, heart, lungs, kidney, spleen, liver, lymph nodes, brain, pancreas, bladder, prostate, GI lesions	All tissues

The index case had a history of travel to an area where Y. pestis is endemic and was transferred to our hospital with signs and symptoms of septicemic plague. He complained of bilateral leg and hip pain. Imaging disclosed multiple lytic lesions and intraosseous abscesses within the entire pelvic girdle, right femur, left tibia, vertebrae and ribs, consistent with multifocal osteomyelitis. Biopsy showed typical features of acute osteomyelitis, and Y. pestis IHC was positive. Abundant Y. pestis antigen was confirmed by IHC in 2 additional cases. In one additional case involving bilateral lower leg amputation, Y. pestis antigen was identified in the submitted necrotic soft tissues, but bone samples were not submitted; associated osteomyelitis is suspected, but cannot be confirmed.

Conclusions: We report a series of Y. pestis cases in which up to 25% of patients were affected by osteomyelitis, a higher incidence than previously described in the literature. Plague patients with skeletal pain should be worked up for osteomyelitis and treated appropriately.

1539 Whole-Genome Sequencing Analysis of blaKPC-Producing Klebsiella pneumonia in Suburban New York City, 2005-2014

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Background: bla_{KPC}-producing *Klebsiella pneumonia*(KPC)was emerging in the mid-2000s and has become the most common carbapenem-resistant *Enterobacteriaceae* in the US patients. Metropolitan New York represents one of the regions with the highest prevalence of KPC worldwide. The aims of this study were to analyze the microbiological characteristics and molecular epidemiology of KPC from patients at a tertiary medical center of New York State over a period of 10 years.

Design: Non-duplicate *K. pneumoniae* isolates from patients at Westchester Medical Center in suburban New York City from 2005 to 2014 were included. Routine identification and antimicrobial susceptibility testing were performed using the MicroScan System. A bla_{KPC}-specific PCR and/or DNA sequencing was used to determine the presence or absence of bla_{KPC} gene. Whole genome sequencing (WGS) was performed on the Illumina MiSeq or HighSeq systems and analyzed with various bioinformatics tools.

Results: From 2005 to 2014, a total of 7,683 non-duplicate *K. pneumoniae* clinical isolates were recovered from patients and examined for carbapenem susceptibilities. Of these, 1,273 isolates were resistant to meropenem (16.6%). The peak prevalence of carbapenem-resistance among *K. pneumoniae* was observed in 2007 (28.4%), which was decreased thereafter with the lowest prevalence of 7.4% in 2014. bla_{KPC} was detected in approximately 95% of carbapenem-resistance *K. pneumoniae* isolates examined. WGS analysis of 213 representative bla_{KPC}-producing *K. pneumoniae* isolates identified 27 known sequence types (ST) and 3 new ST types. Among these, ST258 (n=170, 79.8%) was the predominant clone, followed by ST483 (n=5), ST111 (n=4), ST37 (n=4) and 23 additional STs each with 1-2 isolates. Nosocomial transmission of KPC isolates among inpatients was evident using WGS and epidemiological data. In addition, WGS identified the first KPC isolate in the US with both bla_{KPC} and $bla_{CTX\text{-}M15}$.

Conclusions: The production of bla_{KPC}-carbapenemase is the major mechanism accounting for carbapenem resistance in *K. pneumoniae* among our patients. *K. pneumoniae* ST258 was initially identified and continues to be the predominant clone that might have nosocomial spread among inpatients.

Keywords: *Klebsiella pneumoniae*; bla_{KPC} (KPC), Whole-genome sequencing; carbapenem-resistance *Enterobacteriaceae* (CRE); Carbapenemase.

1540 Return of the "Great Mimicker": Treponema Pallidum in the Gastrointestinal Tract

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Background: Syphilis is caused by the spirochete *Treponema pallidum* and has been called "the great mimicker" because of its propensity to mimic other diseases, both clinically and histologically. In the gastrointestinal (GI) tract, syphilis infrequently causes gastritis and proctitis, but commonly causes perianal condylomata lata, papular skin lesions which occur in intertriginous areas. Clinically, syphilitic gastritis and proctitis may result in mucosal ulcerations, nodularity, masses, and even regional lymphadenopathy—features which may raise clinical concern for inflammatory

bowel disease (IBD) or malignancy. Histologically, the dense chronic inflammatory infiltrate seen in syphilitic lesions can mimic lymphoma, *Helicobacter pylori* infection, autoimmune gastritis, and IBD.
Design: To illustrate the spectrum of syphilitic lesions in the GI tract, we report a series of four patients: one with gastric syphilis, two with rectal syphilis, and one with a condyloma latum.
Results: While the traditional teaching is that the chronic inflammatory infiltrates seen in syphilitic lesions are rich in plasma cells, our experience suggests that the infiltrate is far more lymphoid than plasmacytic (lymphocytic inflammation predominated in all four cases), so looking for sheets of plasma cells may be misleading. Furthermore, other histological features reported in syphilitic lesions were present in only a minority of our patients, such as perivascular cuffs of inflammation (present in 0/4 cases), granulomata (present in 1/4), and prominent endothelium (present in 2/4). In each case in our series, it is particularly notable how the diagnosis was ultimately made: in the first patient because of an unexpected clinical response to *Helicobacter pylori* eradication therapy, despite negative *Helicobacter* serology and lack of response to high dose proton pump inhibitor; in the second patient because of the uncovering of new clinical history; and in the third and fourth patients because the pathologists involved had recently seen similar cases.
Conclusions: As the incidence of syphilis is increasing once again in North America, pathologists must be aware of the wide spectrum of clinical and pathological findings occurring in patients with the disease. We emphasize a practical approach to the differential diagnosis of GI syphilis and describe histological features that should prompt the pathologist to consider syphilis.

1541 Histologic Features and Immunohistochemical Characterization of Inflammatory Infiltrates in Eschar Biopsy Specimens from Patients with Rickettsia parkeri Rickettsiosis

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Background: *Rickettsia parkeri* rickettsiosis is a spotted fever transmitted by the Gulf Coast tick, *Amblyomma maculatum,* and usually found in the southeastern United States. Clinical features include fever, myalgia, maculopapular eruption and eschar formation with symptoms typically milder than Rocky Mountain spotted fever (RMSF). We describe the histopathologic findings and characterize the inflammatory infiltrate using immunohistochemistry (IHC) in a series of skin biopsies of eschars from patients with *Rickettsia parkeri* rickettsiosis.
Design: Skin punch biopsies from 13 patients were evaluated by hematoxylin and eosin (H&E), spotted fever group rickettsiae (SFGR) IHC and PCR for *Rickettsia parkeri*. Quantification of mononuclear infiltrates was accomplished by using an immunoalkaline phosphatase technique and mouse monoclonal antibodies for CD3, CD20, CD4, CD8, CD68 and CD1a.
Results: 13 cases from 2006-2014 with *Rickettsia parkeri* infection confirmed by SFGR IHC and PCR were identified. Patients (12 men, 1 woman) had an age range of 17-62 (median age; 53) and were from 8 states. SFGR IHC showed scattered granular and cytoplasmic staining in mononuclear cells in 13 cases. Four cases had documented clinical presentation of eschar. Most common histologic epidermal features were spongiosis (11), ulceration (9), parakeratosis (8), and lymphocytic exocytosis (6). Most common dermal features were perivascular lymphohistiocytic dermatitis (13), small vessel vasculitis (11) with fibrinoid necrosis (5), interstitial fibrin (8), neutrophilic infiltrates (6), periadnexal inflammation (6), extravasation of red cells (6), interface dermatitis (5), and basal cell vacuolization (5). By IHC, the inflammatory infiltrate in all cases consisted mainly of CD3+ T-cells and CD68+ macrophages. CD8+ cells were more predominant than CD4+ cells. B-cells were <5% of the infiltrate and CD1a+ Langerhans cells were rare in all cases. By histology plasma cells and eosinophils were minor components of the infiltrate.
Conclusions: *Rickettsia parkeri* rickettsiosis is an eschar-associated infection characterized by perivascular and periadnexal lymphohistiocytic infiltrates comprising predominantly CD68+ macrophages and CD8+ T-cells. Dermal neutrophils are found in ~50% of cases. B-cells, Langerhans cells, plasma cells and eosinophils are minor components. These findings are distinct from those typical of RMSF.

1542 Development of an RNA In Situ Hybridization Assay for Hepatitis C Virus (HCV) Detection in Patient FFPE Samples

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Background: Liver biopsy plays an important role for the management of patients with hepatitis C virus (HCV) infection, especially for staging fibrosis. While current HCV identification and genotype classification mainly depend on serum based tests, it is valuable to identify HCV infection in patient liver biopsy samples in parallel with evaluation of the progress of liver fibrosis. Considering the limitation of HCV detection by immunohistochemistry, we hypothesized that RNA *in situ* hybridization may present a valuable method to detect HCV infection in hepatocytes from liver biopsy.
Design: HCV-specific probes were designed based on RNAscope® technology: genotype 1-specific probes (V-HCV-GT1); a pool of probes to detect seven different genotypes (V-HCV-pool). For a preliminary assessment, five patient liver FFPE biopsy samples from patients with known HCV status were tested with these probes by RNAscope in a blinded fashion. After scoring the RNAscope results, the samples were unblinded and results were compared with clinical hematology data.
Results: All five patient FFPE biopsy samples exhibited strong Hs-PPIB signals by RNAscope. HCV-specific RNA signals were detected in four out of five samples where HCV infection had been confirmed by reference methods. One HCV negative sample (P4) by RT-PCR and ELISA resulted in negative by RNAscope with no visible RNA signal. For the four samples from HCV-positive patients, RNAscope results were consistent with HCV infection status and the genotype information derived from RT-PCR. Table 1 summarizes the results.

Table 1, Comparison between RNAscope and reference test data on blood samples

Sample ID	RNAscope scoring			Clinical hematology data			Note
	Hs-PPIB	V-HCV-pool	V-HCV-GT1	Viral Load (lu/ml)	Genotype	HCV Ab (s/co)	
P1	3	1	1	10^5	1b	31.9	-
P2	3	1	1	10^7	1b	20.4	-
P3	3	1	0	10^7	3b	27.8	-
P4	3	0	0	N/A	N/A	N/A	HBV infection
P5	3	2	1	10^6	1b	33.3	Liver fibrosis

Conclusions: The RNAscope® presents a promising method to detect HCV RNA *in situ* either for screening multiple genotypes of HCV or to characterize specific HCV genotype. The preliminary data shows 100% concordance between RNAscope and reference information. A larger study including a larger number of patient samples is being planned to further establish the specificity and sensitivity and its potential clinical utility as a new HCV diagnostics.

1543 NKX3.1 Identifies Fungal Organisms from the Esophagus

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Background: *NKX3.1* is a transcription factor and a prostatic tumor suppressor gene. The principal utility for NKX3.1 has been identifying prostatic adenocarcinomas. On a gastrointestinal biopsy from a patient with a history of prostate adenocarcinoma, we incidentally observed that NKX3.1 stained fungal forms. To the best of our knowledge, it is unknown whether NKX3.1 can be used to identify fungal organisms. We describe novel functionality for NKX3.1 compared to Grocott and PASD on esophageal biopsies.
Design: We identified cases of esophageal biopsies based on the search term "candida" from 3/28/2012 to 12/27/2013. Deeper levels of each case were stained for NKX3.1 (polyclonal rabbit, Biocare Medical, CP422B), PAS with diastase, and Grocott. The tissue fragments ranged from 1 to 6 mm in size. Repeated Measures One way ANOVA (GraphPad Prizm) and Tukey-Kramer multiple comparison tests were used for analysis.
Results: Of 85 cases for which 3 stains were available and at least 1 stain was positive, 83 cases stained as positive with NKX3.1, compared to 79 with PASD and 75 with Grocott. The average number of positive fragments per slide was 2.45 (SEM 0.17) for NKX3.1, compared to 2.33 (SEM 0.18) for PASD and 1.92 (SEM 0.17) for Grocott. Comparing each fragment directly among the three stains, NKX3.1 had on average 47.17 (SEM 2.70) positive organisms, compared to 45.67 (SEM 4.32) for PASD and 38.88 (SEM 2.71) for Grocott. For each of the above comparisons, NKX3.1 was significantly superior to Grocott but not to PASD ($P < 0.05$). Of note, for 3 slides excluded from data analysis, Grocott staining was too weak to interpret, while this was not found for the PASD or NKX3.1 stained slides. In addition, the following stains had suboptimal but interpretable staining: 7 Grocott with weak staining, 1 PASD with weak staining, 1 PASD with dark staining, and 0 NKX3.1.
Conclusions: NKX3.1 was significantly more efficacious in leading to a positive diagnosis of esophageal candidiasis compared to Grocott, resulting in a significantly higher number of positive fragments per slide as well as number of organisms per fragment, but not PASD. Subjectively, NKX3.1 stained round yeast forms superiorly compared to the other two stains, possibly due to its decreased background staining. This added functionality may offset its slightly longer time to stain compared to PASD and Grocott. NKX3.1 will be useful to add to the stain armamentarium for candida and possibly for other fungal species.

1544 Immunohistochemistry Is Rarely Justified for the Diagnosis of Viral Infections

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Background: Viral infections are commonly diagnosed in surgical pathology specimens and account for considerable morbidity and mortality. Characteristic cytologic changes accompany many viral infections (viral cytopathic effect, VCPE), allowing for definitive diagnosis by H&E alone in most cases. Immunohistochemistry (IHC) is often used for confirmation or when clinical suspicion is high but no VCPE is identified. IHC adds to overall cost and turnaround time; however, the utility of IHC in this setting is unknown. In this study, we evaluated the utility of IHC for identifying viral infections and its impact on patient care.
Design: All surgical pathology cases at an academic medical center over a five-year period were electronically reviewed for the use of IHC to detect cytomegalovirus (CMV), herpes simplex virus-1 or -2 (HSV-1/2), varicella zoster virus (VZV), adenovirus, or polyoma virus (i.e., BK or JC). H&E slides from all cases reported as positive by IHC were re-evaluated for VCPE. Patient records were reviewed to determine whether there was a change in treatment as a result of a positive IHC stain in cases without definitive VCPE.
Results: In total, 1636 viral IHC stains were ordered on 1098 specimens from 956 cases. Altogether, 134 (8.2%) stains were positive, including 59/749 (7.9%) CMV, 34/384 (8.9%) HSV-1/2, 16/139 (11.5%) VZV, 3/210 (1.4%) adenovirus, and 22/154 (14.3%) polyoma virus. In 101/134 (75.4%) cases, VCPE was readily identifiable on H&E slides. Of the most commonly encountered specimen types, lack of definitive VCPE was observed in 15/49 (30.6%) cases positive for CMV in the GI tract, 1/10 (10.0%) cases

positive for HSV-1/2 and 2/13 (15.4%) positive for VZV in the skin, and 5/20 (25%) cases positive for polyoma virus in the kidney. In none of the cases without VCPE did a positive finding by IHC result in significant changes in clinical care.
Conclusions: The vast majority of cases for which IHC is ordered for viral infections are negative. A positive IHC stain without diagnostic VCPE is most common for CMV in the GI tract. However, this result rarely impacts patient treatment. These findings suggest that IHC for viral infections without a high degree of clinical or histologic suspicion is unnecessary in the vast majority of cases.

1545 Interpretation of HSV Positive Respiratory Specimens Using Quantitative PCR

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Background: Oropharyngeal reactivation of Herpes Simplex Virus Type 1 (HSV1) is widely recognized to occur in immunosuppressed and critically ill patients. However, it is highly contentious whether the identification of HSV in the lower respiratory tract represents innocuous distal shedding or a true pneumonitis requiring treatment.
Design: The laboratory database was queried for viral cultures conducted between 1/1/14-8/31/15 at a large academic institution with a significant transplant population. HSV positive respiratory viral culture specimens were collected from the archives for HSV genotyping and viral copy number quantification. The published threshold of $>10^5$ copies/ml was considered to be indicative of a clinically significant respiratory viral load. The bronchoscopic findings and associated cytology during specimen collection were reviewed. The medical records of HSV positive patients were evaluated to determine immune status, ICU admission, therapeutic management, and mortality.
Results: 5931 lower respiratory tract viral cultures were conducted, with positive HSV1 viral culture in 46 specimens from 40 patients. PCR results were obtained on 41 specimens from 37 patients. Mean viral copy number was higher in samples collected from ICU vs non-ICU patients ($1x10^7$ vs $1x10^6$, $p<0.05$). There was no statistically significant relationship with mortality or immune status. Diagnostic bronchoscopy and cytology had low sensitivity (36%, 18.8%). Viral cultures had a poor turnaround time (average 10.1 d, range 2-31 d), which diminished their clinical relevance.

Table 1. Bronchoscopy and Cytology Correlation with Viral Count

	+ Bronchoscopy[A]	+ BAL Cytology[B]
Above Threshold (Viral copy $>10^5$)	9/25 (36%)	3/16 (18.8%)
Below Threshold (Viral copy $<10^{[illegible],5}$)	4/9 (44.4%)	0/7 (0%)

A. Viral inclusions or cytopathic effect observed

B. Mucosal ulceration, friability, or edema

Table 2. Immune Status, ICU Admission, Mortality, and Viral Count

	Above Threshold (Viral count > $1x10^{[illegible],5}$ï»¿)	Average Viral Copy #
Immunocompromised	69.5% (16/23)	$2.13x10^6$
Immunocompetent	86.3% (15/18)	$1.45x10^7$
ICU	88.5% (23/26)	$1.13x10^7$
Non-ICU	53.3% (8/15)	$1.08x10^{[illegible],6}$
Death during hospital admission	86.7% (13/15)	$8.93x10^{[illegible],6}$
Survival to discharge	69.2% (18/26)	$6.76x10^{[illegible],6}$

Conclusions: This is the largest US based study using quantitative PCR to evaluate the clinicopathologic significance of HSV isolated from the lower respiratory tract. Quantitative PCR is a specific, sensitive, and rapid test that provides better data for clinicians to treat their patients.

1546 Histopathologic Features of T. Pallidum Infection Differ between the Rectum and Anus

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Background: Syphilis is a sexually transmitted disease, caused by Treponema pallidum, which has become increasingly prevalent. Syphilis not uncommonly manifests in the gastrointestinal (GI) tract, either as a primary or secondary manifestation, but may be overlooked either clinically or histopathologically. Endoscopic lesions of GI syphilis are non-specific, and may consistent of ulcers, fissures, and polypoid masses, which can be mistaken for inflammatory or neoplastic conditions. The classic histopathology of a lymphoplasmacytic infiltrate in the lamina propria may not be reliable. In order for the prompt treatment of GI syphilis and prevention of long-term complications, it is important for the pathologist to consider and recognize this entity. We aimed to evaluate the histopathologic manifestation of GI syphilis to improve diagnostic accuracy.
Design: The surgical pathology file at the Massachusetts General Hospital was searched for cases of syphilitic or treponemal infection of the GI tract. Hematoxylin and eosin slides were evaluated, as was immunohistochemistry (IHC) for T. pallidum. We evaluated and compared the histopathologic features of GI syphilis by site (rectum versus anus).
Results: Three cases of GI syphilis were available for review (distal colon n=2; anus n=1). Patients were male, ages 33 to 52. Review of the clinical data and histopathology was performed. Clinical evidence of syphilis was confirmed by serology. The endoscopic appearance of lesions included ulceration, nodularity, and fissures, with differential diagnoses including inflammatory bowel disease and lymphoma. Histopathologic review revealed that while the classically described pattern of a dense lymphoplasmacytic infiltrate with granulomatous inflammation was seen in the anus, the rectal cases had a dense lymphohistiocytic infiltrate with frequent eosinophils. Features of inflammatory bowel disease were not identified. Abundant spirochetes were identified in the mucosa by T. pallidum IHC in all cases.
Conclusions: The prevalence of syphilis is on the rise, but manifestation in the GI tract may be overlooked due to its nonspecific clinical and histopathologic features. We studied the histopathologic features of GI syphilis, and found an inflammatory response in the rectum that differed from that seen in the anus. We wish to highlight these differences to increase awareness and to provide the pathologist with morphologic clues for this entity.

1547 Evolution of High-Risk Human Papillomavirus Genotypes in Anal Biopsies from HIV-Positive Patients after Long-Term Follow-Up

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Background: Human immunodeficiency virus (HIV)-positive patients are often infected with multiple high-risk human papillomavirus (HR-HPV) genotypes. Multiple HR-HPV infections are associated with development of anal intraepithelial neoplasia (AIN) and squamous cell carcinoma (SCC). While HR-HPV genotypes in HIV-positive adults may change after one year of follow-up, differences in HR-HPV types after longer periods are unknown. We sought to compare genotypes on initial and long-term follow-up anal biopsies from HIV-positive patients to assess factors that may influence their HR-HPV status and risk of developing AIN or SCC.
Design: Institutional records from 1985-2015 were reviewed to identify HIV-positive patients with 2 consecutive anal biopsies obtained at least 5 years apart. Demographics and potential risk factors were collected. Representative lesional tissue was dissected from unstained tissue sections for each case. DNA was extracted and HR-HPV genotyping for 14 types was performed using multiplex PCR followed by signature Tag/Capture probe hybridization. The initial and follow-up genotyping results were compared using Student's two-tailed t-test.
Results: Fifteen HIV-positive patients each with anal biopsies taken at least 5 years apart (mean=9.3 years) were identified, 12 of which had both initial and follow-up tissue available for genotyping. Among the 25 biopsies collected, 12 (48%) were high-grade AIN, 9 (36%) were low-grade AIN or condylomas, and 4 (16%) were SCC. In these biopsies, 76% contained non-HPV 16 and 18 types. Genotyping revealed that one patient was HR-HPV negative on both biopsies. Among the 11 patients positive for HR-HPV, genotype profiles changed from initial to follow-up biopsy in all patients. The mean number of HR-HPV types decreased from 2.8 on initial biopsy to 1.4 at follow-up (p=0.011), but multiple types were detected in 54.5% (6/11) at follow-up.
Conclusions: In the first long-term follow-up study of HR-HPV in anal lesions from HIV-positive patients, we found that in all patients positive for HR-HPV, the genotypes present differed between initial biopsy and follow-up. Most of these lesions contain non-HPV 16 and 18 types which may not be prevented by vaccination. Despite a reduction in number of HR-HPV types at follow-up, the majority of patients still had multi-type infections. Our findings suggest that there is wide temporal variation of HR-HPV genotypes in HIV-positive patients which may contribute to the high risk of developing AIN or SCC in this population.

Informatics

1548 Assessment of Workload Measures in Anatomic Pathology

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Background: Measuring workload in anatomic pathology is a perennial problem because of the large variation of specimen types which require vastly different amounts of time to analyse and report. Systems which have been employed include counting Request, Specimen or Slide numbers and more complex systems including Medicare Relative Value Units (RVU's) and Royal College of Pathologists (RCPath) Workload Points. With increasing sub-specialisation it is essential to find a fair and reliable measure of Workload across sub-specialty Teams to ensure that each of these sections is appropriately resourced.
Design: In our institution Adult Anatomic Pathology and Cytology Requests are prospectively scored at Accessioning and given a RCPath Workload Score and a Local Workload Score using a Modified RCPath Scoring System (which includes more specimen 'bundling' and point caps per Request). Work is distributed in our Department according to the Local Workload Score. A test set of 20,000 consecutive requests was identified and numbers of Specimens, Blocks and Slides were ascertained. These requests were retrospectively scored for Medicare RVU's.
Consultant Pathologists in the department (n=34) were surveyed and asked to score how reasonable their measured workload was per hour on a sliding scale (with 100% being appropriate, <100% being too little work and >100% being too much work). A separate survey could be completed for each team.
These data were analysed by Team and used to calculate a mean appropriate Local Workload Points per unit time. This was then combined with the test set to calculate the appropriate number of Specimens, Slides, RCPath Points and RVU's per Hour.