3A (p=0.8). The correlation between SUV and Ki-67 proliferation index was compared using Parsons test (r=0.35, DF=26, p=0.02).
Conclusions: FDG-PET shows a significant correlation with pathologic grading of FL, but is not a reliable indicator of the Ki-67 proliferation index in these neoplasms. Thus, FDG-PET may provide a useful adjunct to objective grading of follicular lymphomas.

1550 Positron Emission Tomography Reliably Distinguishes Aggressive but Not Low Grade Lymphomas from Reactive Lymph Nodes

G Zheng, M Vuica-Ross, KH Burns, CD Gocke, MJ Borowitz, AS Duffield. Johns Hopkins Hospital, Baltimore, MD.
Background: 18F-fluorodeoxyglucose positron emission tomography (FDG-PET) is widely utilized in clinical management of lymphoma, including pretreatment staging and therapeutic monitoring. In PET imaging, semi-quantitative analyses using standardized uptake values (SUVs) allow for an objective assessment of glucose uptake and metabolic activity. Significant FDG uptake can also be seen in benign conditions, creating diagnostic dilemmas. The aim of this study was to evaluate whether SUVs in FDG-PET studies can be reliably utilized to distinguish reactive conditions, low-grade B cell lymphomas and aggressive B cell lymphomas from one other.
Design: From January 2009 to September 2012, patients with a diagnosis of reactive lymphoid hyperplasia, low-grade B cell lymphoma (follicular lymphoma, mantle cell lymphoma, marginal zone lymphoma, small lymphocytic lymphoma, lymphoplasmacytic lymphoma, and low-grade lymphoma, not otherwise specified), or aggressive lymphoma (diffuse large B cell lymphomas and Burkitt lymphoma), who had received a corresponding FDG-PET scan for initial staging, were identified. SUV values were only utilized from locations where the tumor was biopsy-proven, and the FDG-PET scans were performed within two months of the original biopsy.
Results: 27 patients with reactive lymph node(s) were identified, as well as 32 patients with a low-grade lymphoma and 33 patients with an aggressive lymphoma. Average SUVs are 5.6 (1.2-18.4) for reactive lymph nodes, 5.0 (0-14.3) for low-grade lymphoma, and 14.7 (5.4-31.5) for high-grade lymphoma. High SUV is an excellent predictor for high-grade lymphoma (vs reactive conditions: AUC=0.91 by ROC analysis, p<0.001; vs low-grade lymphomas: AUC=0.93 by ROC analysis, p<0.001). At a SUV cutoff of 7.2, there is a sensitivity of 94% and a specificity of 85% in differentiating a high-grade lymphoma from reactive lymph nodes. However, SUV is not useful in differentiating reactive lymph nodes from low-grade lymphomas (AUC=0.54 by ROC analysis, p=0.59).
Conclusions: Although a high SUV (>7.2) strongly predicts a high-grade lymphoma, the significance of a relatively low SUV is uncertain as this can be seen in both reactive lymph nodes and low-grade lymphomas. In those situations, clinical correlation and possible biopsy are needed.

1551 Day 14 Bone Marrow Cellularity Is Superior to CD34 Immunohistochemistry in Predicting Complete Remission in De Novo AML but Not in Secondary/Therapy-Related AML

G Zheng, A Dezern, KW Pratz, JE Karp, C Gocke. Johns Hopkins Hospital, Baltimore, MD.
Background: The achievement of complete remission (CR) after induction therapy leads to improved survival of AML patients. We previously reported that day 14 bone marrow cellularity is highly predictive of CR in de novo AML but not in secondary/therapy-related AML. In this study we examined the same cohort of patients to evaluate the role of day 14 marrow CD34 immunohistochemistry in predicting CR.
Design: 49 newly diagnosed, previously untreated AML patients received a timed sequential induction regimen of flavopiridol, cytosine arabinoside and mitoxantrone (FLAM) at a single institution. Bone marrow trephine biopsies and aspirates at approximately day 14 of the first cycle of chemotherapy were reviewed and clinical records were abstracted. Immunohistochemical staining for CD34 was performed on routine sections using a standard protocol. Two investigators independently counted 200 cells for CD34 positive blasts.
Results: Mean CD34 count is 21 blasts (range: 1-123)/200 cells for de novo AML, and 25 (range: 2-104) for secondary/therapy-related AML. Interobserver agreement was excellent (Pearson r=0.92, p<0.0001). For de novo AML, low day 14 bone marrow CD34 positive blasts/200 cells is a good predictor for CR (AUC=0.73 by ROC analysis, p=0.04) (Figure 1A), but not as good as cellularity count (AUC=0.98, p<0.0001); for secondary/therapy-related AML, day 14 bone marrow CD34 positive blast count is a poor predictor of CR (AUC=0.56, p=0.69) (Figure 1B), which is similar to cellularity count.

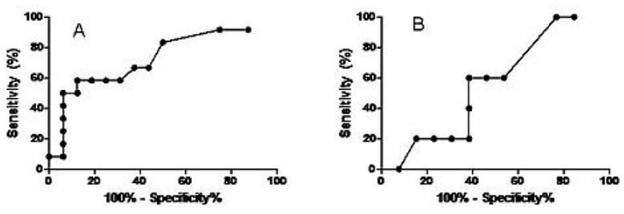

Figure 1

Conclusions: CD34 positive blast count may be helpful in predicting CR when AML is de novo. However, cellularity count is more sensitive and specific as a CR predictor for that cohort. Both cellularity and CD34 positive blast count are not informative in secondary or therapy-related AML. Compared with other measures of remission such as flow cytometry, both cellularity count and CD34 immunohistochemistry are easy and rapid assessments, and they may allow for targeting of de novo AML patients who are unlikely to achieve remission and in need of more aggressive therapy.

Infections

1552 Identification of Fungi in Tissue: A Discrepancy between Histology/Cytology Versus Microbiology

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Background: Core biopsies and aspirates are procedures performed to help diagnose lung nodules and infiltrates. On occasion, a portion of this tissue is sent to microbiology, especially when infection is considered. The histologic assessment includes histochemical and immunohistochemical stains. The microbiology work-up includes direct smears with calcofluor-white (CW), tissue culture, and a 14-day fungal culture. Our goal was to find cases with tissue histology or cytology and corresponding microbial studies, then compare the turnaround time (TAT) and final diagnosis of the two methods of detection. The specificity and availability of the results may be extremely important especially with immunocompromised patients.
Design: We searched the Pathology Laboratory Information System from 1/2009 to 9/2012 for lung core and cytology biopsies with a positive diagnosis for fungi. Subsequently, we obtained corresponding smear, tissue, and fungal culture results from the Microbiology laboratory.
Results: A total of 22 histology/cytology cases were positive: 13 *Aspergillus*; 4 *Rhizomucor*; 1 *Histoplasma*; 2 *Cryptococus*; 1 *Coccidioides*; and 1 *Blastomyces*. Of the corresponding cases in microbiology, immediate direct smear with CW revealed 7 fungi: 4 septate hyphae, 2 aseptate hyphae, and 1 yeast. Subsequent fungal culture results were positive for 3 *Aspergillus fumigatus* (5-day) and 1 (diagnosed as *Blastomyces* on histology) for *Cokeromyces recurvatus* (7-day). The remaining 15 cases showed no growth. TAT was 2 days for histology, while direct smear and positive culture results were reported immediately.
Conclusions: Our study shows a marked discrepancy in detection of fungi between microbiology and histology. Possible reasons include sampling and misinterpretation of contaminants and artifacts on histology. However, this does not appear to completely explain the difference of 7 cases identified by microbiology versus 22 by histologic assessment. Microbial studies are likewise subject to contaminants and technical errors. Although microbiology is necessary for speciating fungi, our results suggest that when given very limited sample, submitting tissue entirely to histology may provide greater yield than microbial studies. Histology also has the advantage of identifying additional findings, e.g. carcinoma, that may be secondarily colonized. A prospective study of a larger series with follow-up, to compare the two methods, is necessary to determine if there is significant advantage or any clinical impact of using one method over the other.

1553 Validation of a Real-Time PCR Assay for Detection of *Pneumocystis jiroveci* in Respiratory Specimens

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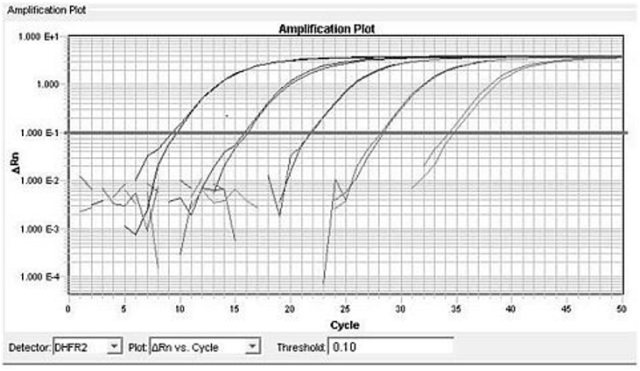
Background: The diagnosis of Pneumocystis pneumonia(PCP) relies on microscopic identification of the stained organism. Due to its instability outside human body, the microscopic identification of *Pneumocystis Jiroveci*(PJ) results in significant number of false negatives. The organism cannot be cultured in-vitro which further complicates the diagnosis. Early diagnosis of PCP affects prognosis. Compared to microscopy, diagnosis by PCR is more rapid & sensitive. The quantitation of fungal load helps to differentiate active pneumonia from colonization. We validated a real-time PCR(RTPCR) that detects & quantifies the PJ load in respiratory specimens.
Design: Thirty-six specimens (34 BAL, 2 sputum) were collected from patients with clinical suspicion of PCP. DNA was extracted by QIAGEN EZ1 Tissue Kit and analyzed for presence of PJ using real-time PCR assay on ABI Prism 7900HT Sequence Detection System. The assay utilized primers and TaqMan probe targeting the PJ dihydrofolate reductase (*DHFR2*) gene region. Each sample was tested in duplicate. Human albumin gene was co-amplified and used as a normalizer to control for the number of cells tested. Quantitative load was reported as PJ copies/100,000 cells. A positive control plasmid containing partial PJ *DHFR2* gene sequence was established from a PJ positive patient sample.
Results: Among 36 specimens, PJ positivity was in agreement between PCR and microscopy in 4 specimens (PJ load $4.4x10^2$ - $3.5x10^6$ copies/100,000 cells) confirming assay's accuracy. The PCR was more sensitive to detect 1 additional low positive PJ (5 copies/100,000 cells) in a sputum sample. The positivity of this sample was also confirmed by 2 other PCR assays targeting different PJ gene regions (*KEX1* & beta-tubulin). The specificity of the assay was tested on selected viral, bacterial & human DNA samples with no cross reactions being observed. The *DHFR2* PCR was linear across 8 orders of magnitude and was sensitive enough to detect 10 copies of PJ on serially diluted positive plasmid sample.



Assay's precision & reproducibility were assessed by testing 2 specimens in different dilutions on 4 runs. The results were in agreement among replicates.
Conclusions: The RTPCR for detection of PJ is rapid, more sensitive, accurate, specific and reproducible.

1554 The Etiology of EBV-Negative Post-Transplantation Lymphoproliferative Disorder Remains Unclear

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Background: While the majority of post-transplantation lymphoproliferative disorder (PTLD) cases are associated with Epstein-Barr virus (EBV) infection, up to 42% are EBV-negative (EBV-N). The antigenic stimulus that drives EBV-N PTLD is unknown. Although hepatitis C virus (HCV), human herpesvirus 8, BK virus and simian virus 40 have all been investigated as possible driving factors in EBV-N, none has been convincingly proven. PTLD and IgG4 syndrome have common features, including plasma cell proliferation driven by chronic antigenic stimulation. We postulated that a subgroup of EBV-N PTLD may be causally related to autoimmune IgG4 disease.
Design: Histology was reviewed on 38 retrievable, formalin-fixed paraffin-embedded cases of PTLD, diagnosed between 1990 and 2012 at the University of Virginia. Cases were classified according to the 2008 World Health Organization Classification. Tissue to evaluate EBV status was available in 33 cases, by immunohistochemistry (IHC) for LMP and/or in situ hybridization for EBER. Plasma cell-rich PTLD were defined as either plasmacytoma/plasma cell myeloma or polymorphous types. IHC for IgG4 was performed on samples containing PTLD and available benign biopsies taken prior to the PTLD diagnosis.
Results: Nine of 33 PTLD cases were EBV-N (27%). The mean time to diagnosis was significantly longer in EBV-N versus EBV-P cases at 86 (6-155) months and 24 (3-103) months respectively (p= 0.02). Three EBV-N patients had available serologic testing for viral infection; two were HCV non-reactive, one had evidence of previous parvovirus B19 (PVB19) infection, and one of the two HCV-negative patients had evidence of a recent PVB19 infection. One of 8 plasma cell-rich PTLD cases was EBV-N. Twenty-eight cases had sufficient tissue to evaluate IgG4 plasma cells by IHC. None of the 28 cases had more than rare IgG4 positive cells. A benign duodenal biopsy from a lung transplant patient, taken six days prior to a biopsy demonstrating duodenal EBV-N PTLD, showed focal staining (a single HPF field with >30 IgG4-positive plasma cells).
Conclusions: Our study serves as a reminder that EBV-N PTLD is more common than is often clinically recognized, and usually occurs late in the post-transplant course. The unknown antigens that play a role in EBV-N PTLD are likely unrelated to those that drive IgG4 disease. Hitherto untested viruses in transplant patients may play an etiologic role in EBV-N PTLD.

1555 The Utility of Frozen Section in the Detection of Invasive Fungal Species in Combat-Related Injury

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Background: Invasive fungal infection is an uncommon but serious complication of traumatic injury. One retrospective review of U.S. military personnel returning from combat operations in Iraq and Afghanistan with combat-related injuries over a six-year period reported an overall incidence of 0.4 cases per 1000 admissions, with a peak in incidence of 5.2 cases per 1000 admissions temporally associated with increased operational tempo in theater. We would like to report our institution's experience with the utility of frozen sections in guiding management of invasive fungal infections in combat-related traumatic injuries.
Design: A review of frozen section evaluations for fungal elements performed on combat-related soft tissue injuries involving U.S. military personnel deployed in support of Operations Enduring Freedom and Iraqi Freedom in Afghanistan and Iraq, respectively, from July 2010 to August 2011 was performed. A total of 147 samples from 48 patients were evaluated intraoperatively for the presence of invasive fungal species using hematoxylin and eosin stained frozen sections, and then later correlated with permanent H&E sections and Gomori methenamine silver (GMS) staining.
Results: Routine permanent and special stains demonstrated fungal elements in 24 of 147 specimens, with 19 of 48 patients having at least one sample positive for fungus on permanent sections. The false positive rate was 1.4% (2/147), and the false negative rate was 10.9% (16/147), with a sensitivity of 60.0% and a specificity of 98.1%.
Conclusions: Invasive fungal infection is a rare but life-threatening complication of traumatic injury often requiring aggressive medical and surgical management. Our experience suggests that while frozen section evaluation can be a helpful adjunct, its low sensitivity makes it suboptimal as a stand-alone intraoperative screening test for fungal elements. We hypothesize that the observed false negative rate is likely attributable to inherently poor staining characteristics of fungal organisms in general, coupled with the known limitations and interpretive difficulties associated with frozen sections. Due to these difficulties, our institution subsequently transitioned from screening for fungal elements via intraoperative consultation to expedited traditional tissue processing with H&E and GMS stained sections with rapid turn-around to ensure optimal, timely management of this unique patient population.

1556 Identification of Burkholderia Cepacia Complex Isolates by MALDI-TOF MS from Burkholderia Cepacia Selective Agar

J Jedrzkiewicz, M Fisher. University of Utah, Salt Lake City, UT.
Background: Despite modern advances in the management of cystic fibrosis patients, *Burkholderia cepacia* remains a troublesome diagnosis. The prognosis is much worse for cystic fibrosis patients colonized with members of the *B. cepacia* complex (Bcc), especially after lung transplantation. Therefore, correct identification of the Bcc is extremely important for appropriate management. The *B. cepacia* complex can be difficult to identify because of its heterogeneous colony morphology and ambiguous metabolic profiles. Specialized media such as *Burkholderia cepacia* Selective Agar (BCSA) is often used to improve the isolation of Bcc, however additional testing is required to confirm an isolate belongs to the Bcc. Confirmatory testing may include extended biochemical panels, 16S rRNA gene sequencing, or more recently, Matrix-assisted laser desorption-ionization time-of flight mass spectrometry (MALDI-TOF MS) analysis. MALDI-TOF MS compares spectral patterns from unknown isolates to a database of known spectra. Previous studies have shown variability in MALDI-TOF identification of bacteria from selective media. Within this study, we evaluated the performance of MALDI-TOF identification of Bcc isolates grown on BCSA.
Design: We examined 31 Bcc isolates previously identified using 16S sequencing. Isolates were plated on Columbia sheep blood agar (SBA) or BCSA and incubated aerobically at 35^{0}C. Isolates were harvested from both media and were prepared using both direct smear and extraction methods. MALDI-TOF scores and identifications were compiled and mean scores compared by the students t-test.
Results: Most isolates scored >2.0, consistent with species-level identification. All samples were identified as species belonging to the *B. cepacia* complex. On average, samples analyzed after extraction yielded higher scores than direct smear samples. There was not a statistically significant difference between scores from SBA and BCSA groups by either sample preparation method.
Conclusions: Our data suggest that *B. cepacia* may be successfully identified directly from BCSA without the need to first subculture to nonselective media.

1557 New Archetypal Poxvirus Infection in an Immunosuppressed Man

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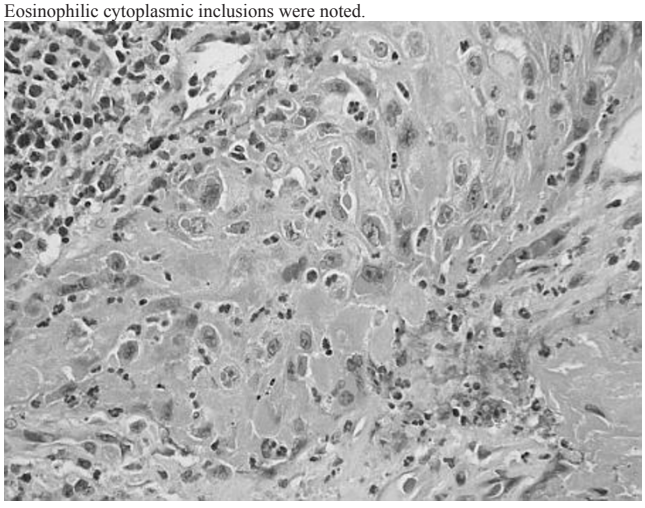
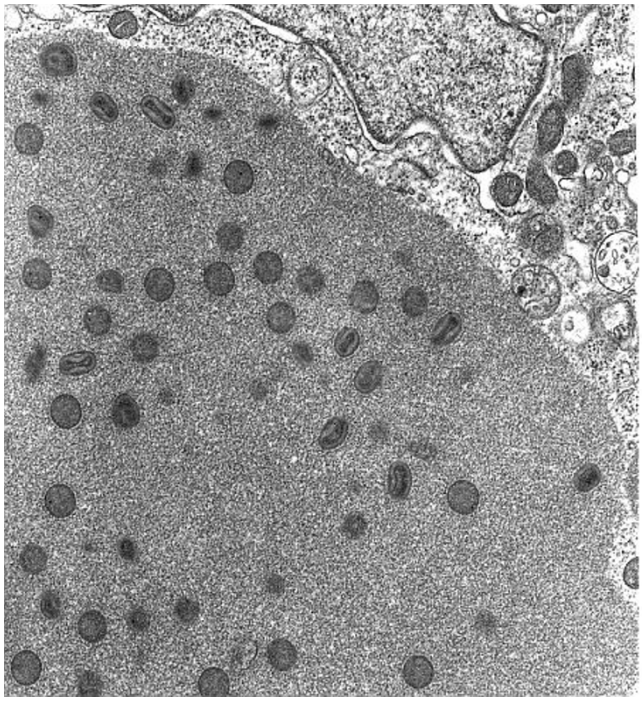
Background: Orthopoxvirus genus includes many species isolated from mammals, such as Variola, Vaccinia, Cowpox, Monkeypox. We report a patient with severe necrotizing skin lesions caused by a recently sequenced novel archetypal poxvirus.
Design: A 45-year old Caucasian man with a stable renal allograft presented with a 3-week history of a rash, which worsened, eventually involving the entire right flank. Multiple debridements revealed the histopathological finding of a dense inflammatory infiltrate of lymphocytes and histiocytes extending into the subcutaneous adipose tissue. Eosinophilic cytoplasmic inclusions were noted.



There was no evidence of Herpes Simplex, Varicella, acid fast bacilli and/or fungi. Further analyses included viral cultures, electron microscopy (EM), and viral genomic sequencing.
Results: Classic cytoplasmic poxvirus A-type inclusions were identified by EM in cultured Hep-2 cells.



Samples sent to the CDC could not be identified by any of the standard PCR primer sets targeting hemaglutinin (HA) or A-type inclusion body protein genes used to speciate orthopoxviruses. Massive parallel sequencing, and de novo assembly, allowed further characterization. The assembled virus genome was 200,058 nucleotides in length, 70.5% Adenosine and Thymidine (A-T), and encoded 201 putative genes. This novel poxvirus has a similar structure to orthopoxviruses both in the number and ordering of the genes in the central region. No HA gene coding region was identified. This virus is not efficiently neutralized by anti-orthopoxvirus serum. The new poxvirus encoded open reading frame DNA sequences (ORFs) not previously annotated in any poxvirus genome. The genome has been sequenced entirely and the poxvirus has since been named Pox_NY014.

Conclusions: This appears to be the first human case of infection with a novel poxvirus that represents an ancestral virus to the orthopoxvirus genus.

1558 Adenovirus Infection in Solid Organ Transplant Recipients

V Mehta, K Mudaliar, PC Chou, MM Picken. Loyola University Medical Center, Chicago, IL; Ann & Robert H Lurie Children's Hospital of Chicago, Chicago, IL.

Background: Adenoviral (AdV) infection in an immunocompromised host carries a mortality rate approaching 80%. It is seen most frequently in bone marrow transplant recipients, but in solid organ transplant (SOT) recipients it usually involves the graft only. Most of the adenovirus disease develops in the first 6 months after transplantation, particularly in pediatric patients. Among abdominal organ recipients, small bowel grafts are most frequently affected, presumably due to the presence of a virus reservoir in the mucosa-associated lymphoid tissue. However, other grafts may also be involved. AdV graft infection leads to graft loss in most instances. Therefore, an awareness of the pathology associated with such infections is important in order to allow early detection and specific treatment.

Design: We reviewed 6 SOT recipients with AdV graft involvement from 2 institutions We sought to compare the diagnostic morphology and the clinical and laboratory findings.

Results: There were 4 females and 2 males with small bowel (x3), heart (x1) and renal transplants (x2, both adults); 4 children (age 2-5) and 2 adults (53 and 61) years. Kidney biopsies were done for renal failure with hematuria; small bowel for diarrhea; clinically stable heart allograft biopsy was done following a prior diagnosis of AdV native kidney nephritis. All patients had AdV (+) serology; stool was (+) in 3 small bowel transplant recipients. Immuno stain (IHC) for AdV was positive in all grafts and in 1 native kidney. In the kidney (graft x2 and native x1), necrosis was tubulocentric with a vaguely granulomatous mixed inflammatory infiltrate associated with rare cells with a cytopathic effect. There was also a lymphocytic infiltrate, simulating T-cell rejection, with admixture of eosinophils. In the small bowel grafts, there was a focal mixed inflammatory infiltrate with associated necrosis. In the heart, allograft AdV was detected by IHC in the absence of necrosis. All patients were subsequently treated and cleared AdV infection, as evidenced by follow-up biopsies, with no loss of the grafts.

Conclusions: AdV infection can involve allografts and native kidney in SOT recipients. Infection is associated with variable necrosis and acute inflammation, in addition to a rejection-like infiltrate. Hematuria in non-renal SOT recipients may be associated with AdV nephritis and clinically-silent graft involvement. Prompt diagnosis (aided by IHC and serology), with specific treatment, can prevent graft loss.

1559 *Helicobacter pylori* 23S rRNA Gene Sequencing for the Identification of Mutations Associated with Clarithromycin Resistance in FFPE Samples

M Mitui, SK Khokhar, NK Leos, CD Doern, JY Park. Children's Medical Center, Dallas, TX; UT Southwestern Medical Center, Dallas, TX.

Background: *Helicobacter pylori* (*H. pylori*) causes gastritis, gastric ulceration, and gastric carcinoma. The antibiotic clarithromycin (CLAR) is commonly used to treat infections with this organism; however, some populations have a high prevalence of CLAR resistance. *M*utations in the 23S rRNA gene of *H. pylori* mediate resistance. Recently, clinical guidelines have recommended testing of 23S rRNA for the management of persistent *H. pylori* infection. *C*linical testing for 23S rRNA in the United States is not widely available. We designed an assay based on PCR amplification and sequencing of the *H. pylori* 23S rRNA gene.

Design: *H. pylori* 23S rRNA testing was targeted to a DNA region that contains mutations associated with CLAR resistance. *H. pylori* sequences from GenBank were used to design *H. pylori* specific primers; however, the true specificity could not be determined because of unavailable sequence information from non-*pylori Helicobacter* species. Cultured *H. pylori* DNA was obtained (ATCC) for optimization of the PCR reaction. B-actin PCR was performed as internal control. 46 FFPE archival samples from 33 cases were evaluated. These samples had been previously evaluated by *H. pylori* immunohistochemistry (IHC). *H. pylori* load in each FFPE sample was assessed by counting all organisms present on a single IHC slide tissue level.

Results: All FFPE tissues with ≥48 organisms per IHC tissue were detected by PCR and sequencing. By sequencing, 63% of cases had the known resistance mutation (2143A>G) in the heterozygous or homozygous state. A nucleotide substitution (2131C>T) of unknown significance was identified in 1 case. In 3 FFPE samples with IHC organisms counts per tissue level of ≤23, there was no *H. pylori* PCR amplification. Furthermore, we examined 5 samples from 4 cases of *H. heilmannii*. Only 2 samples had ≥48 organisms per tissue level and these were both positive for *H. pylori* 23S rRNA PCR; sequencing for mutation evaluation is pending.

Conclusions: In this ongoing study, the CLAR resistance mutation (2143A>G) was identified in 63% of *H. pylori* cases. Indeed, recent guidelines consider >20% to be a high resistance rate requiring the use of non-CLAR regimens. This DNA sequencing test can be implemented for the clinical evaluation of single cases or for epidemiologic studies to determine the prevalence in various communities. Future studies will examine the specificity of this assay for *H. pylori* versus other *Helicobacter* species such as *H. heilmannii*.

1560 Immunohistochemical and Genetic Evaluation of EBV-Positive Aggressive B Cell Lymphomas

DP O'Malley, KE Grimm, R Agrawal, LM Weiss. Clarient Inc./GE Healthcare, Aliso Viejo, CA.

Background: Diffuse large B cell lymphoma and related entities are the commonest type of non-Hodgkin lymphomas. There are several distinct clinicopathologic groups. Among these are plasmablastic lymphoma (PBL), lymphomatoid granulomatosis (LyG) and 'EBV positive large B cell lymphoma of the elderly' (EBV-LBCL-E). Each of these subgroups is rare and immunohistochemical and genetic characterization has been based on only small series. We evaluated a series of 380 large B cell lymphomas for EBV expression and compared these cases.

Design: 380 cases were evaluated for EBV expression by EBER staining. In addition, an extensive panel of immunohistochemical stains (CD20, CD3, CD5, CD10, cyclin D1, bcl6, bcl2, EBER, Ki67, CD30) and a panel of FISH studies (including MYC, IGH/bcl2 and bcl6) were performed on these cases.

Results: 23 cases of EBV-LBCL (6%), 4 cases of PBL (1%) and 4 cases of LyG (1%) were identified. Demographic findings for EBV-LBCL included 21% of cases identified in patients below the age of 50 years. IHC findings in EBV-LBCL included a low percentage of CD10 expression (11%), with high levels of MUM1 (90%), BCL2 (87%) and CD30 (100%). The majority of cases were of non-germinal center (non-GC) type by Hans classifier, with all cases non-GC-type by tally classifier. Nineteen of 23 cases had FISH studies with 26% having BCL6 translocations.

Conclusions: Our study shows the frequency, immunophenotypic and genetic findings in EBV positive LBCL to be comparable to other literature reports. We found a high frequency of non-GC type lymphomas, with a relatively high frequency of BCL6 translocations. We also found that EBV-LBCL-E may be a misnomer, as these cases appear in substantial numbers in patients below 50 years of age.

1561 Evaluation of the Becton Dickinson Phoenix Yeast Panel and the RapID Yeast Plus System in the Identification of Yeast and Yeast-Like Organisms

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Background: The frequency of opportunistic yeast and yeast-like infections continues to grow as the prevalence of immunocompromised individuals rises from transplantation, HIV/AIDS, neoplastic diseases, and immunosuppressive therapies. These infections are associated with high morbidity and mortality rates, especially as the diversity of the organisms continues to expand and drug resistance becomes more prevalent. Therefore, the ability to make a rapid and accurate diagnosis is essential. In 2011, a new automated system, Becton Dickinson (BD) Phoenix Yeast Panel, was introduced. It uses fluorogenic and chromogenic substrates for yeast identification. Manufacturing studies claim greater than 95% accuracy for all the 64 yeast and yeast-like isolates. This study compares the new methodology with a common manual qualitative micromethod, RapID Yeast Plus System, which uses conventional and chromogenic substrates for identification. To our knowledge, this is the first known independent study to evaluate the BD Phoenix Yeast Panel.

Design: Clinical yeast isolates collected at a single institution from March to August 2012 were plated to Sabouraud-dextrose Agar and incubated at 30°C for a maximum of 48 hours. Cultures were suspended into Phoenix or RapID yeast inoculation broth after 24 and 48 hours, respectively and processed. In addition to this, ChromAgar analysis and germ tube test were performed for additional phenotypic characterization. Discrepant results were retested with the API 20C AUX and results were obtained after 72 hours of incubation.
Results: A total of 133 isolates were obtained from 14 different clinical species with the four most common species being *C. albicans* (27%), *C. glabrata* (26%), *C. tropicalis* (20%), and *C. parapsilosis* (13%). The Phoenix and RapID systems agreed for 127 isolates (95%). Of the 6 discrepancies, API 20C AUX agreed with the Phoenix for 5 isolates and the RapID Yeast Panel for 1 isolate. The 5 misidentified by the RapID yeast system were all of the same species, *C. parapsilosis* and overall the RapID panel only identified 12 out of 17 *C. parapsilosis* isolates (70%). Overall, no statistically significant difference was observed between the BD Phoenix and RapID Yeast Plus systems (P=0.09, Chi Square test).
Conclusions: The two identification systems produce similar results. However in this study, the BD Phoenix yeast panel appears to identify *C. paraspilosis* more accurately. Molecular confirmation of the isolates and correlation of discordant results would provide further explanation for discrepancy.

1562 The Detection and Identification of Yeasts from Formalin-Fixed Paraffin-Embedded (FFPE) Tissues Using the Broad Fungal Assay on the PLEX-ID PCR-Electrospray Ionization Mass Spectrometry (ESI-MS) System

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Background: Diagnosis of yeast infections is typically accomplished by fungal culture, morphologic examination of affected tissues, and/or serologic studies. Although histology or cytology analysis is often successful in providing a definitive identification, results may be indeterminate when only few yeast forms are present or morphologic features are ambiguous. This is problematic when cultures are not submitted or fail to yield a causative agent. Newer molecular-based assays may be useful in these situations for providing a diagnosis or confirming the morphologic impression. The purpose of this study was to evaluate the PLEX-ID Broad Fungal Assay to detect and identify yeasts directly from FFPE tissues.
Design: The accuracy of the PLEX-ID Broad Fungal assay was compared to culture and histopathology results for 80 tissue specimens containing a variety of yeasts. Histopathologic results were confirmed by 2 independent reviewers. For the PLEX-ID test, a 40 micron section of the FFPE tissue block was digested with proteinase K, followed by extraction and PCR amplification of DNA using broad-range fungal primers. Amplified products were identified using electrospray ionization mass spectrometry. Discordant results were resolved by D2 rRNA sequencing.
Results: PLEX-ID analysis resulted in 71.3% (57/80) agreement with culture results to the species level with 6.3% (5/80) discordant results and 22.5% (18/80) not detected by the system. Four of the discordant results yielded organisms that may be considered environmental contaminants while one clinically important discordant result occurred (*Blastomyces dermatitidis* by culture and *Cryptococcus neoformans* by PLEX-ID). Sequencing was negative on the DNA extract from the discordant sample. Lack of detection by the PLEX-ID did not correlate with organism load by histopathology. The majority of histology results (93.7%, 75/80) correlated with culture. In the remaining 6.3% (5/80), the PLEX-ID was able to identify the organism that grew in culture.
Conclusions: The PLEX-ID Broad Fungal Assay is capable of identifying fungi directly from FFPE tissues and can help in the diagnosis of difficult cases or confirm the histologic impression where fungal culture may not have been performed.

1563 Surgical Lung Biopsy in Immunosuppressed Pediatric Patients

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Background: Surgical lung biopsies are typically performed in critically ill, immunosuppressed patients when less invasive techniques and broad spectrum antimicrobial treatments have failed. The differential can include infection, graft versus host disease, post transplant lymphoproliferative disease, recurrent or metastatic disease, drug reaction or radiation pneumonitis. Previous reviews of surgical lung biopsies in immunocompromised pediatric patients were performed in the 1980-90s and revealed rates of treatable etiologies in 54-82% of patients, with pneumocystis accounting for up to 70% of infections. Because of advances in treatment since that time we reviewed our series of lung biopsies to determine a more recent diagnostic yield for this procedure and to determine how well surgical pathology could identify organisms in comparison to culture.
Design: Of all pediatric surgical lung biopsy specimens from 1994-2012, we selected those from immunosuppressed patients with diffuse lung disease. We reviewed the chart for underlying cause of immunosuppression, radiology results, culture results and survival, and we reviewed all the lung biopsy slides and special stains.
Results: A total of 50 specimens were received from 46 patients; 19 (38%) had one or more types of infection identified. Pneumocystis remained the most common organism (8), followed by fungus (7), and CMV (4), and one case each of M Kansasii, RSV, parainfluenza, and Bacillus. 8 of the 19 infected patients (42%) did not survive the acute illness. Of the patients without identifiable infection with follow up, only 5 of 29 (17%) died of the acute illness (p=0.10). Surgical pathology failed to identify a specific agent detected by culture in three cases: one each of CMV, parainfluenza, and Aspergillus infection. Besides pneumocystis, microbiology cultures failed to detect one case of candida and one unclassifiable fungal organism. Dividing the cases into earlier (1994-2002, n=25) and later (2003-2012, n=25) time periods resulted in no significant differences in identification of organisms. In addition to infectious diseases, 3 cases of PTLD, 1 recurrent leukemia and 3 possible cases of drug reaction were identified. The remaining diagnoses were considered not specific and included diffuse alveolar damage, acute and organizing pneumonia, interstitial pneumonitis, and poorly formed granulomas.
Conclusions: Despite advances in treatment, infections such as pneumocystis and CMV are still among the most commonly identified agents in surgical lung biopsies. Identification of a treatable infection does not imply an improved outcome.

1564 Intracytoplasmic Granulocytic Morulae Counts on Confirmed Cases of Human Granulocytic Ehrlichiosis/Anaplasmosis in the Northeastern United States

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Background: Human Granulocytic Ehrlichiosis (HGE), due to *Anaplasma phagocytophilum,* is an intracellular gram negative coccobacillus, transmitted by the *Ixodes* tick. The organism replicates in the cytoplasmic vacuoles of granulocytes to form microcolonies called morulae. PCR is the gold standard of early diagnosis, but can be time consuming and is not routinely available outside of specialty laboratories. Serologic tests are frequently negative during the first week of illness onset and often require both acute and convalescent titers to confirm a diagnosis. The peripheral smear can be prepared and examined quickly for intracytoplasmic granulocytic morulae, and provides prompt diagnosis so that appropriate therapy can be administered in a timely manner. However, the percentage of cells that contain morula is reportedly low and there are no recommendations to help guide a strategy of examining peripheral smears in an effort to maximize the sensitivity of this simple and commonly available test.
Design: Peripheral smears were examined in 14 confirmed cases of HGE/Anaplasmosis. The cases were confirmed by various methods including PCR (50%), serology (35.7%), and examination of peripheral smear with treatment response (14.3%). All 14 patients had relatively severe non-specific symptoms and required hospitalization. The granulocytes were counted until a morula was identified and the number of granulocytes containing morulae per 100 granulocytes was recorded. The average counts of three pathologists were calculated to determine how many cells should be counted to find a positive result.
Results: Morulae were identified before a count of 100 granulocytes in 11 (78.6%) cases, and between 100-200 granulocytes in 3 (21.4%) cases. All 14 cases (100%) had morulae identified before counting 200 granulocytes. The average number of morulae found in cases with morulae identified before a count of 100 was 3.5, and the average number of morulae in cases identified between 100-200 was 0.37.
Conclusions: Peripheral smears are a useful, cost and time effective tool for diagnosing HGE/Anaplasmosis. In positive cases it is very likely that a morulae will be identified in a count of 100 granulocytes. However, it is recommended that a count of 200 granulocytes be performed to increase sensitivity. This is the first study to provide a quantitative analysis for a recommendation to perform peripheral smear testing in an effort to rapidly and routinely confirm a diagnosis of HGE/Anaplasmosis.

1565 Infectious Diseases Are Frequently Associated with Adult Hemophagocytic Lymphohistiocytosis in a Large Series of Cases

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Background: Hemophagocytic lymphohistiocytosis (HLH) is an inflammatory syndrome that can be divided into primary (familial, pediatric) and secondary (acquired, adult) types. Infectious diseases are commonly reported triggers in adults; however, data are limited to a few series. Immunosuppressive therapy improves survival in the pediatric cases.
Design: Confirmed cases of HLH in adults with documented bone marrow hemophagocytosis in a 10 year-period were retrospectively reviewed in two large academic centers. Pertinent clinical and laboratory findings were examined.
Results: Of 65 cases of bone marrow hemophagocytosis, 36 cases of HLH fulfilled at least 5 of the 8 diagnostic criteria (see table). The cases excluded lacked sufficient data to fulfill diagnostic criteria. Ages ranged from 19 to 85 (median 50), M:F ratio of 1.5:1. Half of the cases were attributed to infection (acute EBV infection followed by bacterial infections and histoplasmosis). In 36% the trigger was unclear. Fever was the most frequent symptom (96%). Elevated ferritin was present in all cases. The overall mortality rate was 62% (mean survival of 34 days) with 74% dying despite specific HLH therapy (see table).

Common triggers of HLH in adults		n	%	Frequency of HLH diagnostic criteria (must meet 5 of 8)	n[2]	%
Infectious	Acute EBV	7	19.4	Fever	30/31	96.8
Subtototal n=18 (50%)	Bacteria [1]	5	13.9	Splenomegaly	16/20	80.0
	Histoplasmosis	4	11.1	Cytopenias of at least 2 lineages	29/35	82.9
	Mycobacterium kansasii	1	2.8	Fibrinogen (<150 mg/dL)	15/36	41.7
	CMV	1	2.8	Triglycerides (>265mg/dL)	15/31	48.4
Malignancy	T cell Lymphomas	5	13.9	Ferritin>500 mcg/L	35/35	100
				Ferritin>10000 [3]	23/35	65.7
Unclear		13	36.1	sIL-2r	4 of 6	66.7
Total		36	100	NK function [4]	2 of 3	66.7

Most frequent underlying conditions				1. *Burkholderia sp, Rothia mucilaginosa, Stenotrophomonas*, others
Crohn's	3	CHL	2	2. Denominator is variable: data not available
Autoimmune	3	MDS	4	3. Reported as more specific in children
Hemodialysis	2	Healthy	6	4. Measured by the 51-Cr release assay
NA	16	Total	36	

Conclusions: This is the largest series of HLH cases in adults. Acute EBV and bacterial infections followed by histoplasmosis were the most frequent diseases identified in this series. Neoplasms and autoimmune diseases accounted for a minority of cases. The institution of specific immunosuppressive therapy did not change mortality rates, unlike what has been demonstrated in studies of the pediatric population.

1566 Extra-Oral Plasmablastic Lymphomas as a Predominant Involvement Pattern in AIDS Patients of an Inner City Hospital

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Background: Plasmablastic lymphoma (PBL) is defined as a rare aggressive lymphoma characterized by a diffuse proliferation of large neoplastic B-cells with predominance of immunoblastic/plasmablastic morphologic features and a plasma cell –like immunophenotype.Originally, PBL was outlined as an aggressive, B-cell lymphoma, primarily occurring in the oral cavity and most frequently arising in association with human immunodeficiency virus (HIV) infection.

Design: From 2004 to 2012, 14 cases of patients diagnosed with PBL were identified. Electronic medical records were used for extraction of demographic data, HIV status, HIV-associated risk factors, laboratory data, PET imaging scan results, pathology reports and flow cytometry reports. Pathology slides with immunohistochemistry stains were reviewed.

Results: Of the 14 patients with PBL, there were 13 men and one woman with an age range of 26-63 years. 13 patients were HIV + (92.9%), and one patient was HIV -. Less than one fourth (21.4%) of patients (3) presented with oral lesions and the remaining 71.5% presented with extra oral disease including anus, colon, rectum (5), stomach (2), liver (1), penis (1), inguinal node (1) and lungs (1). Most of the HIV + patients were on HAART therapy (84.6 %) treatment and one patient was reported as non-compliant. In the large majority of cases, PET scan demonstrated disease involving other organs, with only one patient free of widespread lymphoma. The bone marrow was infiltrated in 6 cases (42.8%). The most common HIVassociated risk factor was men having sex with men (MSM) (42.8%). The single HIV negative patient presented with disease in the oral cavity/gingival, and was the only patient noted to be in remission from the disease.

Conclusions: Contrary to other studies, our series demonstrates that the majority of PBL in HIV positive patients occurred primarily in extra oral and extra nodal sites. The most common extra oral sites were the ano/rectal area followed by the stomach, with single occurrences of cases in the lungs, liver and penis. Most of the HIV positive patients were on HAART therapy, with disease involving other organs and bone marrow. Diagnosis of PBL is challenging, particularly when it arises in unusual extra oral locations and present as an undifferentiated neoplasm. In those cases an appropriate immunohistochemical pattern including plasma cell markers and B-cell markers should help in diagnosis of such tumors.

1567 Non-Classical MHC-1 Class 1b HLA-E Expression Levels Are Maintained in HIV-1 Infected CD4 Positive T Cells

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Background: HLA-E is a non-classical major histocompatibility complex (MHC) class 1b molecule that is expressed on virtually all cells. Binding of the HLA-E ligand to the natural killer (NK) cell inhibitory receptor CD94/NKG2A is sufficient to protect cells from NK cell mediated lysis. Infection of CD4 positive T lymphocytes with HIV-1 leads to significant downregulation of CD4 and classical MHC class 1a HLA-A and HLA-B expression. Although a few studies have reported increased HLA-E expression with HIV-1 infection, this effect is not well characterized. In this study, we sought to determine the effects of HIV-1 infection on HLA-E expression levels in CD4 positive T cells.

Design: Stimulated peripheral blood mononuclear cells from healthy volunteer donors were depleted of CD8 positive T cells and infected with the NL4-3 HIV-1 virus strain at varying multiplicities of infection. Cells were harvested at 2 days, 4 days, 7 days, and 10 days, and flow cytometry was utilized to determine expression levels of CD3, CD4, HIV-1 p24, HLA-A,B,C, and HLA-E. Expression of HLA-E in infected p24 positive cells was compared to levels in uninfected p24 negative cells at each timepoint.

Results: Following infection with HIV-1, significant p24 expression was identified in a subpopulation of CD3 positive T cells with low or negative expression of CD4 (mean peak infection of 9.5% of total T lymphocytes). This subpopulation of p24 positive T cells exhibited persistent downregulation of MHC-1 HLA-A and B beginning at the day 4 timepoint and continuing throughout the remainder of the experiment, relative to the p24 negative T cells (mean 72% downregulation). However, no persistent enhancement or downregulation of HLA-E expression was observed in p24 positive T cells compared to p24 negative T cells. HLA-E expression levels remained constant in HIV-1 infected CD4 positive T cells in contrast to the markedly decreased expression of HLA-A and B.

Conclusions: Review of the literature shows that various studies have come to different conclusions as to whether HLA-E expression is actively enhanced or remains stable in HIV-1 infected cells. In these experiments, our data suggest that HLA-E expression remains stable in CD4 positive T cells following infection with HIV-1 while HLA-A and B expression is markedly decreased. This selective downregulation of the classical MHC-1 class 1a molecules with maintenance of HLA-E expression allows infected cells to escape NK cell mediated lysis. Further studies seek to identify potential viral products that may be involved in the relative stabilization of HLA-E expression.

Informatics

1568 Synoptic Report Generators for Molecular and Ancillary Test Reporting in Anatomic Pathology Laboratory Information System (APLIS)

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Background: Synoptic reporting for cancer has increased the consistency, completeness of reporting, ease of interpretation and enabled searchable results. Many of these are done using embedded synoptic report generators that allow for check box or short field reporting. We sought to develop a similar system for reporting of molecular tests and ancillary tests using a custom-built embedded synoptic report generator in our anatomic pathology laboratory information system(APLIS) (PowerPath™, SunQuest).

Design: We built and implemented custom synoptic reporting- based embedded templates using the worksheet function within our APLIS (PowerPath™) for the different molecular and specialized ancillary tests. Molecular tests included a comprehensive lung cancer (EGFR, ALK, KRAS, BRAF), colon carcinoma reporting templates (KRAS, Microsatellite instability (MSI), BRAF, MSI immunohistochemistry) and T- and B- cell clonality. Ancillary tests included breast carcinoma comprehensive reporting template (ER, PR, HER2 IHC, and HER2 FISH) and UroVysion™ reporting templates.

Results: The embedded worksheet reports had the following advantages of standard reporting: 1) Ability to generate a summary results section at the top of report; 2) ease of reporting with standardization of the reporting; 3) ability to generator specific comments specific to prognosis and implications for therapy for a particular mutation (EGFR, KRAS mutation); 3) generation of quality assurance reports such as rates of positivity for mutations, rate of ER/PR/HER expression relative to degree of differentiation of tumor, correlation of cytology with UroVysion™ findings; 4) creation of a searchable database for items such as all particular mutations and ER/PR/HER2 expression; 5) ability to incorporate free text comment sections for needed flexibility.

Conclusions: Embedded synoptic report generators for molecular and ancillary reporting within an APLIS has improved consistency, accuracy, ease of reporting and interpretation and allowed for easier quality assurance tracking and research applications.

1569 Implementation of a Pathology Teaching Website with Integrated Whole Slide Imaging for Greater Compatibility with Tablets/Smartphones

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Background: Study sets involving digitized virtual slides at our institution had limited viewer flexibility and accessibility, typically requiring either a flash-based web browser or a dedicated slide viewing application. We modified our online Genitourinary Pathology Study Set, complete with digital whole slide imaging, to be viewable on computers and smartphones/tablets with integrated toggles of answers and explanations, while maintaining compatibility with different slide viewing programs according to the user's preference.

Design: Website elements, originally coded in HTML, were simplified to enhance readability on mobile devices while retaining password-protected access. Hyperlinks to virtual slides were modified for slides to automatically open using an installed slide viewer, instead of a new web browser window. Multiple links to each slide were created to provide compatibility with web-based viewers, Aperio ImageScope for computer users and several tablet/phone slide viewers including Aperio ePathViewer, Objective or Wholeslide.