

Design: AML M6a patients with or without 20q- evaluated at our institution were compiled and analyzed for basic CBC, bone marrow, cytogenetic, and clinical survival features using Student t-test or Fisher exact probability test (F-test). The findings were considered to be statistically significant if $p < 0.05$.

Results: From 1079 patients diagnosed with either AML (736) or MDS (340), 2.0% (22/1079) of cases had 20q-. Among 736 AML, 26 were M6a. Six (23%) M6a had 20q-, but only 1 (0.14%) of the non M6a AML had 20q-. Therefore, 20q- was more commonly found in M6a than other types of AML ($p < 0.001$, Odds Ratio=44.8; 95% IC: 24.5-81.7). No differences were observed between M6a with or without 20q- in regards to patient age, gender, hemoglobin, WBC, and platelet counts. Compared to the remaining M6a without 20q-, M6a with 20q- patients were more likely to have lower blast counts (11% vs. 18%) ($p = 0.0237$), but had more erythroid precursors (67% vs. 57%, $p = 0.05$) in a bone marrow with similar cellularity (55% vs. 68%, $p = 0.17$) when compared to M6a without 20q-. The median survival time for M6a with 20q- was slightly shorter than M6a without 20q- (3 vs. 5 mo, $p = 0.22$).

Conclusions: Our study revealed that 20q- was more commonly found in M6a than any other type of AML, but the presence of this abnormality appears to have little impact on the clinical outcomes when compared to M6a without this genomic abnormality.

1421 Detection of Merkel Cell Polyomavirus in Chronic Lymphocytic Leukemia Cells by Fluorescent In Situ Hybridization (FISH).

A zur Hausen, ND Pantulu, CP Pallasch, AK Kurz, A Haug, A Kassem, L Frenzel, S Sodenkamp, HM Kvasnicka, E-JM Speel, CM Wendner. Maastricht University Medical Center, Maastricht, Netherlands; University Hospital Freiburg, Germany; University Hospital Cologne, Germany.

Background: Merkel cell polyomavirus (MCPyV) is detected in approximately 80% of Merkel cell carcinomas (MCC). A number of previous studies have shown that MCC patients are at a significantly increased risk to develop chronic lymphocytic leukemia (CLL) and vice versa. Until recently, clonal integration and truncating mutations of the Large T antigen (LTAg) of MCPyV were restricted to MCC. We have recently reported the presence of the MCPyV in highly purified tumor cells of CLL ($n = 19/70$, 27.1%) (Blood. 2010 Sep 3). Of these, six revealed a novel 246bp deletion in the helicase gene of the large T antigen (LTAg). The presence of MCPyV was confirmed by immunohistochemistry.

Design: Here we aimed to determine the presence of MCPyV by FISH analysis in CLL cells in order to evaluate whether MCPyV was integrated or episomal. For this purpose we performed FISH analysis as previously described (Int J Cancer. 2005 Jun 20;115(3):419-28) using MCPyV genome as FISH probe. We tested 2 of the previously reported MCPyV positive CLL cases (EDTA decalcified bone marrow trephines) and MCPyV positive MCC ($n = 5$). In addition, we tested MCPyV negative tumors, e.g. breast and colon cancers. All tissues were formaline fixed and paraffine embedded.

Results: Specific MCPyV DNA by FISH analysis was detected in the nuclei of MCPyV-positive CLL and MCC cells. In contrast to MCC, the FISH signals of the CLL cases revealed more granular signals. However, the CLL specimens derived from EDTA decalcified bone marrow trephines in contrast to the non decalcified specimens of MCCs. No signals were obtained by MCPyV FISH in breast or colon cancer specimens.

Conclusions: The specific detection of MCPyV in CLL cells further supports our previous report of a possible involvement of MCPyV in a significant subset of CLL. The specific but rather granular nuclear FISH signals in MCPyV positive CLL cells point to an episomal presence of MCPyV in CLL cells. Currently we are optimizing the MCPyV FISH protocol including RNase pretreatments in order to test the granular FISH results and to assess further CLL cases for the presence of MCPyV.

Infections

1422 Detection of Melanin by the Fontana-Masson Silver Stain Is Not Specific for Cryptococcus in Tissue Sections.

JA Bishop, AM Nelson, WG Merz, FB Askin, S Riedel. The Johns Hopkins Medical Institutions, Baltimore, MD; Armed Forces Institute of Pathology, Washington, DC.

Background: With the steadily rising number of immunocompromised patients, it is not uncommon for surgical pathologists to encounter yeast and yeast-like organisms in tissue sections. Correct identification of these organisms is imperative for guiding appropriate therapy. Although fungal culture is considered the gold standard for organism identification, very often tissue samples for cultures either are not submitted or are negative. Although most yeast, yeast-like, and dimorphic organisms have characteristic morphologic features, findings often overlap among these organisms, especially in tissue samples of limited volume. Fontana-Masson (FM), a form of a silver stain for detecting melanin in tissue, has been used and accepted as a relatively specific stain for *Cryptococcus neoformans* in tissue based on few studies with limited numbers and types of organisms. This study was designed to test the value and specificity of the FM by investigating a large collection of tissues with organisms that may mimic *Cryptococcus neoformans*.

Design: Cases of *Cryptococcus* and other organisms that can morphologically mimic *Cryptococcus* were identified in the surgical pathology archives of The Johns Hopkins Hospital and The Armed Forces Institute of Pathology. Cases were included if organism identification was culture-proven and/or if cases were highly diagnostic at the morphologic level.

Results: Overall, FM was positive in 26/46 (57%) cases, with 9/9 *Cryptococcus neoformans*, 1/1 *Cryptococcus gattii*, 7/7 *Coccidioides immitis*, 4/10 *Blastomyces dermatitidis*, 2/2 *Paracoccidioides brasiliensis*, 1/1 *Loboa lobo*, 1/1 *Rhinosporidium*

seeberi, and 1/1 *Chrysosporium parvum* staining. FM was negative in 10 *Histoplasma capsulatum*, 1 *Histoplasma duboisii*, 1 *Sporothrix schenckii*, and 2 cases of the alga genus *Prototheca*.

Conclusions: FM was 100% sensitive, staining all *Cryptococcus neoformans* and *gattii* tested. Low specificity, however, limits the value of the FM stain; only 4 of 10 non-Cryptococcal species tested were negative in all cases. These results need to be confirmed and extended to other isolates and species, but it is clear that many organisms in the morphologic differential diagnosis can be FM-positive. Accordingly, results of the FM stain, especially a positive, should be interpreted cautiously and only in the context of the organism's morphologic and host features.

1423 Prevalence of Herpes Simplex Virus Type-2 Seropositivity in Women with Atypical Recurrent Genital Symptoms.

C Chisholm, A Rao, T Kuehl, P Sulak. Scott & White Memorial Hospital, Temple, TX.

Background: Herpes simplex virus type-2 is identified in 17% of people in the United States between the ages of 14-49. Women with atypical recurrent genital symptoms (itching, burning, skin fissures, erythema) represent a population which is frequently misdiagnosed or ignored. Since those with HSV-2 have a two to four times higher risk of acquiring and transmitting HIV, it is prudent to determine if HSV-2 is the etiology behind these abnormal symptoms.

Design: A retrospective review was performed of all HSV-2 and HSV-1 antibody assays ordered during a 12 month interval. Data regarding demographics, reasons for ordering, and antibody prevalence were extracted from medical records without patient identifiers and reported as percentages for various subgroups of women with 95% confidence intervals. Groups with and without genital symptoms were compared using Chi-square test.

Results: HSV-2 antibodies were detected in 30% of 290 women between 14 and 64 years of age undergoing screening without symptoms including patients with screening directed by a healthcare provider (29.6%) or requested by the patient (30%). The frequency of HSV-2 positive test results were significantly ($p = 0.004$) greater in those with the occurrence of atypical recurrent clinical symptoms (47% of 88 patients). Women with atypical recurrent genital symptoms and positive HSV-2 test results were more likely ($p = 0.038$, Chi-square test) to also be positive for HSV-1 (69% of 26) than those with negative HSV-2 test results (41% of 29).

Conclusions: The frequency of HSV-2 positivity in central Texas women screened for various reasons was greater than a general mixed gender population. The frequency of positivity was greater in women with atypical recurrent genital symptoms compared to those screened as part of routine patient care, and the prevalence was equal to those patients with a history of genital herpes. Women with recurrent genital symptoms are thus a high risk population which warrant screening and prophylactic treatment to prevent the spread of HSV-2.

1424 Spectrum of Liver Pathology and Laboratory Findings in HIV Patients with and without Hepatitis C Coinfection.

JA Eisenstein, MG Lim, MM Coates, MB Mosunjac. Emory University, Atlanta, GA.

Background: Liver abnormalities are common in HIV patients. The reported prevalence and the impact of HIV/HCV coinfection vary significantly among studies. The aim of this study was to evaluate histological, clinical and laboratory findings in HIV patients of an inner-city hospital, with and without Hepatitis C (HCV) coinfection.

Design: Clinical and pathologic data was obtained on 168 HIV-positive patients with liver biopsies (LB) from the years 2005-2010 at Grady Memorial Hospital. A retrospective search of medical records categorized patients by age, sex, biopsy indication, pathologic diagnosis and hepatitis serologies.

Results: Patients age ranged from 22-69 (mean 46.5) years with a 2.4:1 male to female ratio. 137 patients were co-infected with HCV. Of the 137 patients, 44 were additionally coinfecting with Hepatitis B (HBV). We identified 31 HCV seronegative patients, 8 whom were co-infected with HBV. The remaining 23 patients had HIV only and had LBs for various clinical and laboratory indications including elevated liver enzymes.

	ALT [IU/L] range; mean; SD	ALT [IU/L] range; mean; SD	# Stage 3 and 4 (%)
Hep C+, Hep B- (N=93)	13-304; 70.7; 61.3	13-466; 71.5; 64.3	25 (26.9)
Hep C-, Hep B- (N=23)	11-203; 92.5; 66.8	17-613; 183.4; 187.1	4 (17.4)
Hep C+, Hep B+ (N=44)	20-398; 74.9; 70.3	21-888; 101.1; 138.1	15 (34.1)
Hep C-, Hep B+ (N=8)	23-507; 120.5; 160.1	29-914; 197.3; 296.4	4 (50)

Biopsy results for the patients coinfecting with HCV demonstrated an overall higher level and incidence of fibrosis. There was one case of hepatocellular carcinoma, one case of ductopenia, and a case of metastatic large-cell neuroendocrine carcinoma from the lung.

Biopsy results for patients who were HCV-seronegative demonstrated the following: Non-specific hepatitis (12), B-virus-related hepatitis (8), two of which were cirrhotic, AFB+ granulomas (2), non-specific AFB- granulomas (2), increased iron stores suggestive of hemochromatosis (2), Kaposi's sarcoma (1), involvement by CLL/SLL (1), primary biliary cirrhosis (1), benign liver cyst (1), and unremarkable liver parenchyma (4).

Conclusions: The incidence of coinfection with HCV in HIV patients of an inner-city hospital is high (81.55%). Coinfected patients show higher degree of fibrosis and cirrhosis however; there were no significant difference in liver enzyme levels than in those patients with HIV alone. Surprisingly, overall, only a small proportion of LB contained malignancy or opportunistic infection and there were no biopsies that would suggest drug-associated reaction.

1425 Chlamydia Trachomatis (LGV) Proctosigmoiditis without Adenopathy Syndrome Mimics Inflammatory Bowel Disease.

M Gallegos, DP Bradly, AS Tenorio, A Keshavarzian, S Jakate. Rush University Medical Center, Chicago, IL.

Background: There has been an increasing prevalence of LGV (lymphogranuloma venereum) or *Chlamydia trachomatis* cases in Europe and North America in the MSM (men having sex with men) population. These cases may present with incomplete history and proctosigmoiditis without characteristic adenopathy syndrome. Such cases may be confused clinically and morphologically with inflammatory bowel disease (IBD). This causes diagnostic delay and worsening morbidity. We describe two such cases initially diagnosed as IBD and subsequently identified as *Chlamydia trachomatis* proctitis.

Design: 2 males (ages 28 and 30) who presented with at least 3 weeks of bloody diarrhea underwent colonoscopy with biopsies. 1 male (age 28) with persistent anal bleeding had a biopsy from an anal verge ulcer. All patients had subsequent (2 to 20 weeks later) endoscopic evaluation. Their clinical profile, travel history, endoscopic findings, serum antibodies and DNA probe assay for chlamydia and initial and subsequent biopsy tissue were reviewed.

Results: All patients had initial clinical and endoscopic suspicion of IBD. Histologically, the colonic and anal biopsies showed patchy chronic inflammation with cryptitis and lymphohistiocytic infiltrates reminiscent of Crohn's perianal disease and IBD-type proctosigmoiditis. None had adenopathy syndrome at presentation to evoke clinical suspicion of *Chlamydia trachomatis*. Subsequent history revealed that all patients were MSM. 1 patient had travel history to Europe but the diarrhea was considered too long standing for an infectious etiology. At the time of initial biopsies, the serum antibody studies were not performed. Since the symptoms persisted after IBD therapy, further studies were performed that included serum antibody and DNA probe assays for chlamydia which were positive. The patient with travel history to Europe subsequently also showed HIV positivity and Kaposi's sarcoma in the cecum. All patients responded to antibiotic therapy for chlamydia.

Conclusions: Patients with *Chlamydia trachomatis* who present without classical history or symptoms and have isolated proctosigmoiditis may be confused clinically, endoscopically and histologically for IBD. When there is lack of response to IBD therapy and/or the clinical profile raises the potential for *C. trachomatis* infection, serological and DNA probe studies for chlamydia can be performed. The specimen must also be sent to the state health department. Awareness of its mimicry to IBD and prompt identification of *Chlamydia trachomatis* are crucial for treatment and prevention of further complications from this disease.

1426 Fatal Acute Meningitis: Pathology and Etiologic Diagnosis.

J Guarner, L Liu, J Bhatnagar, T Jones, M Patel, M DeLeon-Carnes, SR Zaki. Emory University, Atlanta, GA; Centers for Disease Control and Prevention (CDC), Atlanta, GA.

Background: Fatal acute bacterial meningitis has decreased significantly due to vaccinations and early diagnosis and treatment. We studied the pathology of fatal acute bacterial meningitis cases sent to the CDC in order to define the frequency of the different bacterial infections encountered in the last 10 years and associated pathologic features.

Design: The data base was searched for all cases from 2000 to 2009 with a diagnosis of meningitis or meningoencephalitis. Of 118 cases, 29 had acute inflammation in the meninges and demographic, pathologic, immunohistochemical (IHC) and molecular data was assessed. Excluded cases had meningoencephalitis with mononuclear inflammation (58 cases), granulomatous inflammation (21), meningeal hemorrhage (7), or no slides available for review (3).

Results: There were 11 pediatric and 18 adult patients from both the U.S. (27) and Taiwan (2). *Streptococcus pneumoniae* was the cause of meningitis in 16 patients and *Neisseria meningitidis* in 8 patients. The following bacteria were found in one case each: *Streptococcus suis*, *Streptococcus mitis*, *Staphylococcus aureus* and *Fusobacterium*. In one case Gram positive cocci were present but no specific etiologic agent could be determined. The age of the patients with *N. meningitidis* was lower (mean 15.6 years, range 7 to 29) than the age (33.9 years, range 1 week to 68 years) for the patients with meningitis due to other bacteria. The table shows the frequency of different pathologic findings and the usefulness of the different tissue-based diagnostic modalities. Other features included 3 (17%) cases with *S. pneumoniae* that had meningeal hemorrhages and 2 (25%) cases with *N. meningitidis* that had leukocytosis.

	Total	Severe meningeal inflammation	Parenchymal inflammation	Positive Gram stain result	Positive Steiner stain result	IHC positive	PCR positive
Streptococci	18	14 (78%)	8 (44%)	5 (28%)	7 (47%)	17 (94%)	15 (83%)
Neisseria	8	6 (75%)	3 (37%)	2 (25%)	2 (28%)	7 (87%)	7 (87%)
Other	3	3 (100%)	3 (100%)	3 (100%)	1 (33%)	1 (33%)	1 (33%)

Conclusions: *S. pneumoniae* continues to be the most frequent cause of fatal acute bacterial meningitis followed by *N. meningitidis*. Infiltration of the inflammation into the superficial cortex was frequent in these cases. Gram stains were positive in one third of cases while silver stains had higher positive rates. Organism specific diagnosis was possible in 96% of cases with acute meningeal inflammation using IHC and PCR.

1427 Human Herpesvirus – 7 Related Fatal Hemophagocytic Syndrome: A Case Report and Review of Literature.

H Guo, DP Hartmann, M Ozdemirli. Georgetown University Hospital, Washington, DC.

Background: Hemophagocytic syndrome (hemophagocytic lymphohistiocytosis, HLH) is a rare disease caused by a dysfunction of cytotoxic T cells and natural killer (NK) cells. It can be either primary due to an underlying genetic defect, or secondary associated with malignancies, autoimmune diseases or infections. Infectious triggers

are most commonly due to viral infections mainly of the herpesvirus, with Epstein-Barr Virus (EBV) being the most common virus. Other herpesviruses, with exception of Human Herpesvirus-7 (HHV-7), have been reported to be associated with HLH. HHV-7 is a CD4 T lymphotropic herpesvirus with strong homology to HHV-6. The association of HHV-7 with human disease has not been recognised although some cases of exanthem subitum have been linked to it.

Design: 8 pathology specimens from 5 patients with clinical diagnosis of HLH were analyzed for the presence of HHV-7 DNA. DNAs were isolated from the peripheral blood and formalin-fixed tissues. The sequence of HHV-7 was examined by the nested polymerase chain reaction (PCR) technique. The PCR products from positive cases were purified and sent to capillary electrophoresis analysis for HHV-7 sequence by an ABI sequencer.

Results: HHV-7 was detected in one case. The 2 year-old patient presented with fulminant liver failure, splenomegaly, lymphadenopathy and anemia. A liver transplant was performed and explanted liver revealed fulminant hepatitis with extensive bridging necrosis and numerous lymphocytes and histiocytes infiltrate portal and periportal area. Hemophagocytic cells were observed in bone marrow aspirate. Viral screenings for EBV, CMV, Parvovirus B19, Norovirus, enterovirus, adenovirus and HHV-6 in peripheral blood were negative by RT-PCR. HHV-7 sequence was detected in peripheral blood and in formalin-fixed explanted liver tissue by nested PCR, and confirmed by ABI sequencer. Immunohistochemistry staining for HHV-7 was positive on the formalin-fixed explanted liver tissue.

Conclusions: Although more data are needed, the current result provides first evidence that HHV-7 may also play a pathogenic role in HLH as other Human Herpesviruses. In unexpected fatal hemophagocytic syndrome patient, special viral screening for HHV-7 may be critical for ensuring recovery prior to the occurrence of a life threatening complication.

1428 Human Papillomavirus Infection Is Not Associated with Dysplasia and Squamous Carcinoma of the Conjunctiva in a Cohort of Young Patients in South Africa.

MJ Hale, S Naidoo, SM Muthabeni. University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa.

Background: Conjunctival dysplasia and squamous carcinoma are associated with exposure to ultraviolet radiation. Typically, these changes are seen in older patients who have lived for extended periods in tropical regions. The disease has been associated with human immunodeficiency virus (HIV) infection and there is evidence that the demographic profile of patients with this condition is becoming younger, particularly in sub-Saharan Africa as a consequence. It has been suggested that conjunctival infection by human papillomavirus (HPV) may be associated with dysplasia and squamous carcinoma, but there is conflicting evidence for this.

Design: The archives of the Division of Anatomical Pathology at Chris Hani Baragwanath were searched for all conjunctival biopsies in 2007 and 2008 showing dysplasia or squamous carcinoma. Patients 35 years or younger were identified and their pathological changes stratified according to severity of dysplasia and invasive carcinoma. Evidence of HPV infection of the conjunctiva was then sought in this cohort of young patients using the polymerase chain reaction to amplify the L1 region of the HPV genome which yields a 150bp product using the GP5⁺ / GP6⁺ primers, which are generic to all HPV subtypes. PCR amplification for HPV was also performed on conjunctival biopsies from 10 patients showing no evidence of dysplasia as a control group.

Results: A total of 418 patients with conjunctival biopsies were found, 116 being aged 35 years or younger. Of this group, 51 (44%) showed evidence of dysplasia or invasive carcinoma stratified as follows: 5 patients had mild dysplasia, 13 moderate dysplasia, 26 severe dysplasia / carcinoma in situ and 7 invasive squamous carcinoma. Dysplastic changes were commoner in the female gender, 34 (66%) as compared to the male, 17 (34%). There was no evidence of HPV infection in any of the 51 patients with dysplasia or squamous carcinoma, or in the 10 patients without dysplasia.

Conclusions: Increasing numbers of younger patients are presenting with conjunctival dysplasia and squamous carcinoma of the conjunctiva in South Africa, particularly in the female gender. Furthermore, there is no evidence to support the notion that human papillomavirus virus plays an aetiological role in this disease in the region. This finding does not exclude the possibility that HIV infection contributes to the neoplastic process either as a sole initiator or in association with an as yet unidentified infectious agent.

1429 Eosinophilic Abdominal and Hepatic Abscesses in Kurdish Iraq: An Emerging Disease Largely of Uncertain Cause.

H Hassan, R Majid, A Farris, J Guarner, M Hughson. Shorsh General and the University Teaching Hospital, Sulaimaniyah, Iraq; Emory University, Atlanta, GA.

Background: Regional conflicts and the urban migration of rural populations are altering disease patterns of in many countries of the Middle East. Sulaimaniyah in the East Kurdish region of Northern Iraq has experienced an outbreak of eosinophilic abdominal abscesses that were not seen prior to 2009. Previously, eosinophilic liver abscesses have gained little attention in the medical literature.

Design: Eosinophilic abdominal abscesses diagnosed in Sulaimaniyah's Shorsh General and the University Teaching Hospitals in 2009 and 2010 were studied together with patient clinical data.

Results: Five male and 8 female patients had a median age of 27 (range: 1.5-56 years). All lived in urban Sulaimaniyah. Clinical findings included right subchondral pain, fever, malaise, and blood eosinophilia. None had pulmonary symptoms. Stools were negative for ova and parasites. The abscesses were lined by a granulomatous reaction with giant cells surrounding central necrosis and an eosinophilic exudate containing Charcot-Leyden crystals. Ten patients had hepatic abscesses that were localized multicentric 3-5cm lesions in 7 patients and diffuse approximately 0.5-1 cm lesions in 3 patients. One

patient, a 1.5 year old child, had a 3 cm anterior abdominal wall abscess without apparent liver involvement. Four patients had intestinal obstruction due to cecal (2 patients), hepatic flexure, or gastric abscesses. Features suggestive of basidiobolomycosis were found in a patient with cecal and hepatic abscesses. Ova resembling those of Fasciola were found in liver granulomas of another patient. In eleven specimens, no organisms could be seen. In the study period, 19 hydatid liver cysts, were surgically removed. Eosinophilic abscesses were not found in patients with hydatid disease. In the two other large cities of the Kurdish region, one eosinophilic liver abscess was seen in Duhuk in 2010 but not before, and none have been seen in Erbil.

Conclusions: Since 2009, eosinophilic abdominal abscesses, predominantly of the liver, have emerged as a previously unrecognized disease in the Eastern region of Northern Iraq. Although in most cases an etiologic agent has not been identified, parasites and fungal infections are suspected. They are presently seen with more than half the frequency but do not overlap with hydatid liver cysts, the major clinical parasitic disease of Iraq.

1430 Usefulness of Gram Stains for Identification of Morphologically Similar Fungi in Tissue Sections and Cytologic Preparations.

AA Kelly, RE Villanueva, TE Davis, AS Burris, SD Allen. Indiana University, Indianapolis.

Background: Fungi cause significant morbidity and mortality. Microscopic features are useful in differentiating fungi, but several commonly encountered species share similar appearances making definitive identification difficult. Atypical morphology of some fungi can also lead to errors in diagnosis. Gram's stain, when used in combination with other special stains, can aid in making diagnoses. However, differences in the results of tissue Gram stain methods can lead to ambiguity or cause diagnostic errors. The primary object of this study was to evaluate the usefulness of Gram stains as a means of differentiating between commonly encountered fungi in both paraffin sections of tissue and in cytologic preparations.

Design: Paraffin-embedded tissue specimens from infections caused by species of *Candida*, *Histoplasma*, *Blastomyces*, *Cryptococcus*, *Aspergillus*, and *Zygomycetes* were studied. In Part one, we compared the following four different tissue Gram stain procedures: an instrument procedure (IP, Artisan™, Dako), Brown-Hopps (BH), Brown and Brenn (B&B), and a modified Hucker and Conn (H&C) manual Gram stain (Sigma-Aldrich®). Included in Part two of the study were cytologic specimens from the last 2.5 years that were all analyzed using Diff-Quik, Giemsa, GMS, calcofluor white and Gram stains. Identities of fungi were confirmed by microbiologic cultures or DNA probes.

Results: The modified H&C and BH procedures showed consistently reliable staining of *Candida* (14/14). The B&B (13/14) and IP (12/14) procedures were less reliable. *Cryptococcus* also showed consistent positive staining (5/5) with the modified H&C. *Histoplasma* (0/13) and *Blastomyces* (0/6) consistently failed to stain with the modified H&C method, but less consistently with the other Gram stain methods. In cytologic preparations, all *Candida* species (20/20) were stained dark blue. Again, *Histoplasma* (0/10) and *Blastomyces* (0/3) did not retain the crystal violet stain. *Candida* pseudohyphae stained strongly blue with the Gram stain (10/10); *Aspergillus* and *Zygomycetes* (0/8) stained irregularly or not at all.

Conclusions: Gram's stain aids in differentiating both yeast-like and filamentous fungi. Species of *Candida* and *Cryptococcus* yeasts as well as *Candida* pseudohyphae stain intensely dark blue (positive) with the Gram stain while other common pathogenic yeasts and filamentous fungal hyphae do not.

1431 Disseminated Fungal Infections at Autopsy: A Review of 59 Patients in a Single University Medical Center with Detection of Multiple Fungal Species Confirmation by In Situ Hybridization.

C Louie, LA Litzky, A Carruth-Griffin, I Nachamkin, KT Montone. University of Pennsylvania, Philadelphia.

Background: Disseminated fungal infections are an important cause of death in immunosuppressed patients; however fungal infections are not always suspected and culture confirmation is not always possible. We performed an analysis of 59 autopsies with disseminated fungal infection including clinical history, histologic findings, and the involved fungal pathogens identified by histology, culture and/or in situ hybridization (ISH) using species/genus specific probes.

Design: Files were searched for autopsies with disseminated fungal infections from 1996-2010. Clinical information, reports, slides and fungal pathogens were reviewed. In addition, ISH using species/genus specific biotin-labeled oligonucleotide probes targeting *Aspergillus sp.*, *Zygomycetes*, *Candida albicans*, *Fusarium sp.*, and *C. immitis* were performed in cases with and without positive cultures.

Results: 59 patients with multi-site fungal infections were identified. Disseminated fungal infections were suspected in only 31 patients (53%). Underlying immunosuppression was observed in 58 patients (20 transplant, 13 malignancy, 11 steroids, 3 AIDS, 5 liver disease, 6 other). 17 (29%) had multiple reasons for immunosuppression. Sites involved included lung (52), GI/liver/pancreas (27), heart (26), GU tract (25), spleen (14), thyroid (12), and CNS (11). 41 patients had histologic evidence of a single fungus while 19 patients had multiple fungi (17: 2 fungi, 2: 3 fungi). 46 patients (78%) had positive fungal cultures of which 29 (49%) grew a single pathogen (14 *Aspergillus sp.*, 9 *Candida sp.*, 2 *C. neoformans*, 1 *C. immitis*, 2 *Rhizopus sp.*, 1 *Cunninghamella sp.*); 17 (29%) grew multiple fungi (commonly *Aspergillus sp.* or *Candida sp.* and another fungus). 13 patients (22%) had negative cultures. ISH confirmed *Aspergillus* in 16/22 culture positive cases, *Zygomycetes* in 4/4 culture positive cases, *C. immitis* in 1 culture positive case, *C. albicans* in 5/6 culture positive cases, and *Fusarium sp.* in 1 culture positive case. In addition, ISH identified fungi in 7/13 culture negative cases (3 *Aspergillus sp.*; 3 *Zygomycetes*, 1 *Candida sp.*)

Conclusions: Disseminated fungal infections are often clinically unsuspected and multiple fungal organisms may be identified in a large percentage of cases. The lung is the most frequent tissue site involved followed by the heart, GI tract, and GU tract. *Aspergillus sp.* and *Candida sp.* are the most common isolates in our experience. ISH can aid in fungus identification in cases with negative cultures.

1432 Characterization of an Lgr5-Positive Stem Cell Population in Gastric Cancer Tissues and Background Gastric Mucosa.

D Ma, Y Yao, A Ziober, AR Selpulveda. U of Pennsylvania, Philadelphia.

Background: Lgr5 (Gpr49), is an orphan G protein-coupled receptor that is expressed in epithelial stem cells in intestine and stomach, and in cancer tissues, marking cancer stem cells. The role of gastric stem cells in *H. pylori* (HP)- associated gastric cancer is not well understood. In this study, we examined the expression of Lgr5 in gastric mucosa and gastric cancer tissues to determine whether the Lgr5-positive stem cell population is related to the type of mucosa (antral vs. oxyntic), *H. pylori* infection status, and gastric cancer subtypes.

Design: Twenty non-neoplastic and 17 tumor tissue blocks from the same patients were selected from a retrospective cohort of gastrectomy specimens. The specimens were grouped based on the presence or absence of gastric adenocarcinoma (GC) and *H. pylori* status: HP negative patients without GC (HP-/GC-), HP negative patients with GC (HP-/GC+), and HP positive patients with GC (HP+/GC+). The latter two were subdivided into mucosa with no tumor in the block or with tumor. *H. pylori* status and Lgr5 expression were determined by immunohistochemical staining with specific rabbit polyclonal antibodies. The intensity of Lgr5 stain was graded as: 0, no stain; 1, intermediate stain; and 2, strong stain. The overall percentage of positivity (stain grades 1 or 2) was scored. The neck and deep glandular components were graded separately. P values < .05 were considered significant.

Results: Lgr5 was significantly more expressed in the antrum than in oxyntic mucosa, with focal expression scores up to 14.0 ± 1.8 and 2.0 ± 2.2, respectively (p=.005). Significantly higher Lgr5 expression was seen in HP+/GC+ mucosa (n=8) compared with the HP-/GC+ group (n=12) (13.8 ± 3.0 and 6.5 ± 1.5, respectively, p=.031), with a 4-fold increase in the neck region (p=.02). Lgr5 expression was present in 70.6% (12 of 17) carcinomas. Expression of Lgr5 was rare in moderately differentiated adenocarcinoma areas as compared to poorly differentiated areas (1 of 6 vs. 12 of 15, p=.007). Lgr5 expression was higher in tumors from the HP-/GC+ group vs. the HP+/GC+ group (median: 5.0 and 0.0, respectively, p=.03).

Conclusions: The Lgr5-positive epithelial stem cell pool is expanded during *H. pylori* gastritis. The population of Lgr5-positive gastric stem cells appears to be associated with poorly differentiated adenocarcinomas, both in HP-positive and HP-negative stomachs. These data support the notion that Lgr5-positive cells represent a population of gastric cancer stem cells and may be important targets of *H. pylori*-associated gastric carcinogenesis.

1433 Pulmonary Granulomatous Infections: Comparison of the Yield of Cultures and Histologic Examination in 88 Cases from 10 Institutions.

S Mukhopadhyay, C Farver, LT Vaszar, O Dempsey, H Popper, H Mani, VL Capelozzi, J Fukuoka, K Kerr, EH Zeren, V Iyer, T Tanaka, I Narde, A Nomikos, D Gumurdulu, S Arava, DS Zander, HD Tazelaar. SUNY Upstate Medical University, Syracuse, NY; Cleveland Clinic, OH; Mayo Clinic, Scottsdale, AZ; Aberdeen Univ Med School, United Kingdom; Med Univ of Graz, Austria; Penn State Hershey Med Ctr, Hershey, PA; Univ of São Paulo, Brazil; Toyama Univ Hosp, Japan; Çukurova Univ, Adana, Turkey; AIIMS, New Delhi, India.

Background: Histology and cultures are the main methods for detecting organisms in pulmonary granulomas. However, the relative yield of these two methods is not well defined. The aim of this study was to compare the yield of histology with that of cultures for the detection of various organisms in pulmonary granulomas.

Design: Cases of pulmonary granulomatous inflammation from 10 institutions (4 US, 6 non-US) in 7 countries were retrospectively reviewed and classified as infections if an organism was identified by histology or cultures, or if serology was strongly suggestive. In cases where culture results were available, the yield of cultures was compared to that of histology.

Results: Organisms were identified in 125/500 pulmonary granulomas. Results of cultures were available in 88 cases, including cultures of biopsied lung tissue in 35 and of other respiratory tract specimens in 53. Mycobacteria were detected by cultures in 47 cases and histologically in 22. In contrast, only 7 fungi were detected on cultures whereas 31 were detected by histology.

Comparison of Yield of Cultures and Histology for Detecting Organisms in Pulmonary Granulomatous Infections

Organism	Total cases	Culture data available	Cultures pos, Histology neg	Cultures neg, Histology pos	Both pos
Histoplasma	18	13	0	13	0
Coccidioides	13	11	0	6	5
Cryptococcus	6	4	0	3	1
Pneumocystis	2	2	0	2	0
Aspergillus	4	2	1	1	0
Paracoccidioides	6**	0	0	0	0
Fungus, unknown type	2	0	0	0	0
M. tuberculosis	28	28	17	1*	10
Non-tubercular mycobacteria	21	21	15	1*	5
Mycobacteria, unknown type	23	5	0	5	0
2 organisms	2	2	0	0	2
ALL ORGANISMS	125	88	33	32	23

*identified by PCR of fresh biopsy tissue; **diagnosed serologically

Conclusions: In pulmonary granulomas, cultures are more likely to detect mycobacteria than histology, whereas the reverse is true for fungi. Although *Coccidioides*,

Cryptococcus and *Aspergillus* may grow in cultures from pulmonary granulomas, *Histoplasma* and *Pneumocystis* are highly unlikely to do so; their detection, therefore, mostly depends on histologic examination.

1434 Bacterial 16s rRNA Gene Sequencing in the Routine Diagnostic Laboratory.

JD Pimentel, LP Samuel, RJ Tibbetts, L Whitely, SM Dubedat, ELN Dodds. Henry Ford Hospital, Detroit, MI; Royal Prince Alfred Hospital, Sydney, NSW, Australia; Sydney University Prince Alfred Macromolecular Analysis Centre, Sydney, NSW, Australia.

Background: Sequencing DNA for bacterial identification has become more practical due to automated genetic analyzers and alignment software. The literature suggests that identification of bacterial isolates by 16S rRNA gene sequence is more accurate than commercial biochemical kits and should be used to identify any clinically significant isolate with less-than-excellent commercial identification. However, debate has arisen regarding the selection of databases used for identification. Our aim was to evaluate the accuracy of the free databases available with the online BLAST program and evaluate the utility of sequence-based identification in a routine diagnostic laboratory.

Design: 44 isolates (aerobes, anaerobes, mycobacteria) with known identifications (18 ATCC, 26 patient samples) from two different institutions were evaluated. DNA was extracted from isolated colonies using commercial spin columns or by 10 minutes at 95°C. A monochrome (SYBR green I), real-time PCR was used to amplify and detect 500 base pairs of the 16S rRNA gene using either a Rotor-Gene RG-3000 (Corbett) or a SmartCycler 16 (Cepheid). BigDye Terminator 3.1 chemistry (Applied Biosystems,) and either a 3100 or 3730xl DNA analyzer (Applied Biosystems) was used to sequence the real-time PCR product. A BLAST match was then performed with the following parameters: Database nucleotide collection (nr/nt); Exclude models (XM/XP) and uncultured/environmental sample sequences; Optimize for highly similar sequences (megablast). Matches with the highest max scores were evaluated for clinical relevance (e.g. non-human pathogens excluded) and reputable source (reference strain or published biochemical characters). Close matches were further evaluated by comparison of biochemical characters.

Results: Sequence alone provided genus-level identification in 98% of isolates. Species/species complex/species group-level identification was achieved in 93%. There was no differentiation between the genera *Leclercia* and some *Enterobacter spp*; *Enterococcus durans* (ATCC 6056) and *E. faecium*; or *Bordetella bronchiseptica*, *B. pertussis*, *B. parapertussis* and *B. holmesii*.

Conclusions: The methods and results described were reproducible and practically applied in two different routine diagnostic laboratories using different types of equipment. Moreover, use of the free databases provided reliable identifications for pure colonies with a completed biochemical profile.

1435 Kikuchi's Lymphadenitis: Role of Parvovirus B-19, EBV, HHV-6, and HHV-8 Detected by PCR in a Multi-Institutional Review of 18 Cases.

FGN Rosado, Y-W Tang, RP Hassarjian, C McClain, BY Wang, CM Mosse. Vanderbilt University Medical Center, Nashville, TN; Massachusetts General Hospital, Boston; New York University, NY.

Background: Kikuchi's lymphadenitis (KL) is an uncommon self-limited disorder that typically manifests as painless cervical lymphadenopathy and systemic symptoms, usually in young females. The clinical manifestations and pathologic findings suggest a viral etiology, yet specific etiologic agents remain unknown. While there are studies reporting a positive association of KL to Parvovirus B19 and herpesviruses, other studies have failed to find an association between these viruses and KL. To our knowledge, this current study is the largest study of KL in Western patients that used PCR testing for 4 different common viral pathogens.

Design: Archival material from 3 institutions were included, following confirmation of the diagnosis of KL by two pathologists. The age and sex-matched controls selected were comprised predominantly of negative staging lymph nodes in patients with papillary thyroid carcinoma. PCR from the paraffin-embedded tissue sections for Parvovirus B19, EBV, HHV6 and HHV-8 was performed.

Results: Eighteen cases of KL were analyzed, 12 of which (60%) were cervical lymph nodes. There were 14 females and 4 males (3.5:1); 13 (72%) were patients younger than 31 years of age (range 11-41). All the cases showed typical geographic necrosis with abundant apoptotic debris, although the degree of necrosis was variable. PCR revealed a high prevalence of parvovirus B19 in the controls (44%), whereas there were fewer positive cases seen on the KL cases (11%) (p=0.068). There were no significant differences between cases and controls tested for EBV, HHV-6 and HHV-8 (p=0.5).

Kikuchi's lymphadenitis (n=18): PCR results for Parvovirus B19, EBV, HHV-8, and HHV-6								
	Parvovirus B19		EBV		HHV-8		HHV-6	
	KL	C1	KL	C1	KL	C1	KL	C1
POSITIVE	11%	44%	6%	11%	0	0	6%	11%
NEGATIVE	89%	56%	94%	89%	100%	100%	94%	89%

Conclusions: PCR failed to reveal a positive association between KL and 4 common suspected viral agents. These findings do not support a known infectious agent in the pathogenesis of this disease.

1436 Pathologic Studies of Fatal Enterovirus 71 Encephalitis in the United States.

W-J Shieh, J Bhatnagar, CD Paddock, SR Zaki. Centers for Disease Control and Prevention, Atlanta, GA.

Background: Enterovirus 71 (EV71) is a member of the human enterovirus species A in the family of *Picornaviridae*. It is one of the etiologic agents of hand-foot-mouth disease (HFMD) and has been associated with neurologic disorders, such as aseptic meningitis, encephalitis, myelitis, and a syndrome of acute febrile paralysis similar

to poliomyelitis. Since the late 1990s, outbreaks of EV71 encephalitis with severe neurologic complications or fatal outcome among young children have occurred in many Asian countries. In the United States, only sporadic cases or localized small outbreaks of EV71-associated illnesses have been reported and very few fatal cases have been studied by postmortem examination. We present pathologic studies on a series of U.S. fatal cases of EV71 encephalitis in this report.

Design: From 1997 to present, 933 cases of central nervous system infection of unknown etiology were submitted to the Infectious Diseases Pathology Branch at CDC for evaluation. Eight fatal cases caused by EV71 from 5 different U.S. states were identified and included in this study. Autopsy tissue samples of these cases were evaluated by histopathologic examination, immunohistochemical assay (IHC), and molecular techniques.

Results: The median age of the patients in this study was 2 years, ranging from 10 months to 6 years. The most prominent histopathologic findings included neuronal necrosis, neuronophagia, glial nodules, and inflammatory infiltrates in parenchyma, perivascular areas, and meninges. Histopathologic changes were most severe in the brainstem and spinal cord. Viral antigens were usually observed by IHC in infected neurons, neuronal processes, glial nodules, and areas of necrosis. The presence of EV71 RNA in the central nervous system was detected by a nested-RT PCR assay targeting the VP1 gene of EV71 and sequencing of amplicons.

Conclusions: EV71 has emerged as an important pathogen in recent years, causing multiple outbreaks of HFMD with severe neurological complications among young children in the Asia-Pacific region. Its role in central nervous system infections is not well recognized in the U.S. because of relatively low incidence. Nevertheless, EV71 is an important etiologic agent of acute meningoencephalomyelitis, with high rates of morbidity and mortality in pediatric population. Our studies highlight the histopathologic features of fatal EV71 cases and underscore the importance of performing IHC and RT-PCR assays on autopsy samples. More studies are needed to further understand the pathogenesis of EV71 infection.

1437 Polyomavirus Sinusitis.

HN Spartz, L Miravalle, SD Allen. Indiana University, Indianapolis.

Background: Viral diseases are a significant source of morbidity and mortality, particularly in immunosuppressed individuals. The well-characterized human polyomaviruses, BK and JC virus, have long been known to cause serious illness in immunosuppressed patients including nephropathy, hemorrhagic cystitis, and progressive multifocal leukoencephalopathy. More recently, three additional polyomaviruses have been found to naturally infect humans; KI, WU, and MC viruses. Polyomaviruses are thought to be transmitted via a respiratory route at an early age resulting in subclinical or mild respiratory illness. The viruses subsequently establish latent, asymptomatic infections in various tissues in immunocompetent patients. Immunosuppression allows reactivation from a subclinical state to lytic infection and severe or fatal disease. The recently described KI and WU viruses have been associated with respiratory infection in immunocompetent and immunosuppressed children and adults. However, their role in respiratory disease remains unclear.

Design: In this study, we examined sinus mucosa from a 57 year old patient with a long-standing history of CLL/SLL and known secondary immunosuppression, including a history of CMV colitis. The patient presented with massive periorbital edema, fever, and sharp pain over the sinuses. A CT of the sinuses showed nearly complete opacification of the maxillary, sphenoid, and frontal sinuses. Endoscopic sinus surgery was performed.

Results: Routine H&E sections revealed numerous sinus epithelial nuclei distended with pale, eosinophilic, homogenous inclusions and margined chromatin. Immunohistochemical studies were negative for EBV, Herpes 1 and 2, Adenovirus, and CMV. No bacterial, fungal, or acid-fast microorganisms were detected. Immunohistochemistry using the anti-SV40 monoclonal antibody (MRQ-4, Cell Marque, Rocklin, CA) directed against the SV40 polyomavirus large T antigen demonstrated intense positive staining of the nuclear inclusions. Electron microscopy of the nuclear inclusions revealed abundant, densely packed, uniform, unencapsulated viral particles measuring approximately 35 nm.

Conclusions: The current study demonstrates the first reported case of sinusitis with polyomavirus infection. The case supports the proposed route of polyomavirus infection and demonstrates a possible role for polyomaviruses in upper respiratory infections in immunosuppressed patients. Additional studies are underway to further identify and sequence the virus.

1438 Are Routine Anaerobic Blood Cultures Necessary?

AT Vischio, A Pandit, J Seifert, L Ovittore, S Tittle, JL Dodge. Danbury Hospital, Danbury, CT.

Background: There has been a decline in the percentage of positive blood cultures yielding anaerobic organisms according to the medical literature. In order to assess whether or not it would be clinically safe and cost effective to draw only aerobic bottles during routine blood culture draws, we analyzed our laboratory data. Correctly diagnosing sepsis can have favorable results for the patient and also positive financial results for the hospital. A typical case of severe sepsis costs \$25,000 per patient, corresponding to approximately \$17 billion annually. If patients progress to septic shock and multiple organ dysfunction expensive therapeutic, diagnostic interventions and longer hospital stays would be required. The average cost of a positive anaerobic blood culture is \$17.37 which includes \$2.87 per bottle, \$7.50 for technician time and \$7.00 to run a confirmation panel on the Siemens MicroScan WalkAway 96 Plus®.

Design: From January 2010 to May 2010, microbiology laboratory records of all blood cultures (3,769) were analyzed from both outpatient and inpatients at our institution.

Results: Organisms were isolated from 257 of 3,769 sets (positivity rate 6.8%): Forty-eight (48) were determined to be contaminants (1.9%). Therefore, 209 were considered

clinically important. Of these, 17 (8.1%) were detected during anaerobic incubation and 43 (20.8%) were detected during aerobic incubation. The rest of the isolates (150) were detected during both aerobic and anaerobic incubation.

(Table 1) Isolates That Only Grew In Anaerobic Blood Culture Bottles

Facultative Anaerobes	Obligate Anaerobes
Lactobacillus sp. (2)	Bacteroides sp. not fragilis (1)
Staphylococcus aureus (2)	Bacteroides fragilis (1)
Escherichia coli (4)	Bacteroides fragilis species (1)
Pseudomonas aeruginosa (1)	Peptostreptococcus (1)
Streptococcus agalactiae (1)	
Enterococcus faecalis (1)	
Escherichia fergusonii (1)	
Citrobacter freundii (1)	

Conclusions: It would not be clinically safe or cost effective to eliminate anaerobic blood culture bottles. We would have missed 17 clinically important bacteremias (8.1%) that could have harmed patients and led to overall higher costs. In this study, predominately facultative anaerobes were isolated from the anaerobic blood culture bottles (13 of 17). The BD BACTEC Lytic/10 Anaerobic/F Culture Vials system used at our institution utilizes .26% saponin, a detergent that lyses red and white blood cells, enabling the recovery of some bacteria that would not have grown in aerobic culture bottles.

Informatics

1439 Evaluation of Microvessel Density Using Light Microscopy Versus Computerized Image Analysis in Multiple Myeloma.

N Aboud, TW Kelley, JR Jacobsen, S Tripp, A Wilson, R Fenn, SL Perkins, ME Salama. ARUP Reference lab, salt lake city; University of Utah, Salt Lake city; St. Jude Children's Research Hospital, Memphis.

Background: Emerging data suggest a prognostic value of angiogenesis in bone marrow in Multiple Myeloma (MM). However, most of studies used a quantitative evaluation of the microvessel density using a method, so-called hot-spot technique that was developed for characterization of angiogenesis in solid tumors. Given that solid neoplasms and the bone marrow have differences regarding the vascular structures and the distribution of microvessels, we evaluated performance of microvessel count done manually on light microscopy (MVP) and microvessel parameters obtained using computerized image analysis in the context of other prognostic and microenvironment factors.

Design: MM cases diagnosed in 2007-2008 with adequate materials were included in the study. Immunohistochemical stains for CD138 and CD34 were performed to highlight Plasma cells (PC) and vasculature, respectively. Adequately CD34 & CD138 stained bone marrow core biopsies were digitally scanned with the ScanScope[®] XT system (Aperio Technologies, Inc., Vista, CA) and areas with most abundant PC were analyzed via 'microvessel density algorithm. Parameters obtained from the algorithm including microvessel density (MVD), vessel perimeter (VP), total number of vessels (TV) and segments with vessels of various bin areas (0-15, 15-30, 30-50, 50-75, 75-100, and 100 μ m² increment up to 900 μ m²) were analyzed with regards to association / prediction of PC and other prognostic / microenvironment parameters. All statistical analyses were performed using SAS software, Version 9.1 of the SAS System.

Results: 46 patients (29 M) ranged in age between 41-78 y were included. Immunoglobulin subtypes and microenvironment parameters including mast cells & trabecular volume didn't correlate with PC. Microvessel density as measured by MVP, MVD, or TV, correlated significantly with the PC with p values of <.0001, 0.0018 and 0.006, respectively. MVP showed higher Pearson ranking as compared to VP and TV at 0.56, 0.45 and 0.40, respectively. MVD was the most tightly correlated parameter with disease stage. When ranked for predictive power for plasma cell number, the segment of blood vessel with cross-area of 400-500 μ m² ranked higher for prediction of PC.

Conclusions: Both MVP and MVD significantly correlate with plasma cell number. MVP was the highest predictor according to Pearson correlation. MVD correlated better with the stage of the disease. Vessels within 400-500 μ m² segment ranked the highest in predicting plasma cells number.

1440 Google Earth and Panoramic Photo Software in the Management of Virtual Slides.

L Alfaro, E Poblet, MJ Roca, P Catala, A Navea. Fundacion Oftalmologica del Mediterraneo, Valencia, Spain; Albacete University, Spain; Hospital Arnau de Vilanova, Valencia, Spain.

Background: Virtual slides are high resolution scanned images obtained from glass slides, and stored in a multi-layered pyramidal file format to allow review at computer screens with quick "zoom in" and "drag" along the image, simulating an optical microscope. Virtual microscopy has greatly developed; however, high prices of scanners and software have hindered a much broader implementation. We have tested the use of free and low cost software to overcome these limitations. Google Earth and diverse panoramic photo software share with virtual microscopy the fact of using image zooming and dragging. They seem suitable to be adapted for virtual slides.

Design: Ten slides from the files of our hospital with samples of different size and shape were retrieved. Two scanners were used to obtain virtual slides, a Mirax Midi (Zeiss) and an Aperio XT. Scanned files with .mrxs and .svs specific extensions were exported to conventional .tiff and .jpg files. New images with pyramidal structure were generated for Google Earth, Zoomify, HD View, Silverlight Deep Zoom, and Gigapan. Images were hosted in two servers, a commercial hosting server (<http://e-pat-org/vs/COMP>), and a portable USB hard drive with Apache server software.

Results: All five software options allowed the display of the virtual slides without limitations for diagnostic purposes. Some advantages and minor differences between

programs were mainly found regarding conversion processes from the original slides. Google Earth showed a good display, but a slower pyramidal structure creation when using big size samples (i.e. 2 x 2 cm) with the generators used (Map Tiler, and Google Earth Photo Overlay Creator). Zoomify, which is based on Flash, had a slightly less soft transition between fields, but a higher compatibility, and an excellent viewer to compare two different images. HD View had the fastest converter, and a very good quality, but a specific plug-in was required. Silverlight Deep Zoom had also a clean transition between fields but the converter (Deep Zoom Composer) showed incompatibilities with .tiff files using JPEG compression. The major limitation of Gigapan is that files have to be hosted at Gigapan server.

Conclusions: Free and low cost software for virtual microscopy is available with no need of high computer knowledge, and is highly suitable to establish compatibility between different virtual microscopy devices.

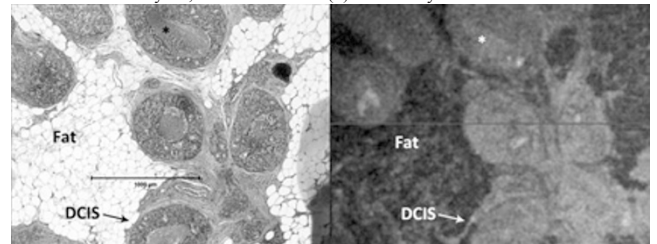
1441 Direct Digital Imaging of Breast Tissue Using Spectral-Domain Optical Coherence Tomography.

JL Fine, L Kagemann, G Wollstein, H Ishikawa, JS Schuman. University of Pittsburgh, PA.

Background: Spectral-domain optical coherence tomography (OCT) permits direct imaging of tissue in 3D, potentially bypassing glass slide workflow. Other specialties are developing in-vivo microscopic imaging using OCT and other techniques. This represents a challenge and an opportunity; we can begin augmenting traditional histology and pathology with 3D-derived information. Here we correlated OCT images with H&E images of breast tissue.

Design: OCT images were acquired from formalin-fixed, paraffin-embedded tissue blocks representing normal breast, papilloma, DCIS, invasive ductal carcinoma, and axillary lymph node with small tumor deposits (Bioprogen, Research Triangle, North Carolina, USA). 2 mm deep volumes of tissue were sampled with 500 x 500 x 1024 voxel resolution over small areas of the block (transverse resolution 20 microns, axial resolution 2 microns). OCT images underwent post-processing then were correlated with routine H&E stained slides. Virtual slices, 3D reconstructions and animations were created.

Results: Features such as fat vacuoles, vessels, and tissue outlines were readily identified, as were some normal ducts. Ducts involved by DCIS were easily recognized when surrounded by fat; comedo necrosis (*) was faintly visible.



Benign breast ducts and lobules were subtle, seen as slight shadows in the image with a larger duct structure. Invasive carcinoma was also subtle but had a discernable texture. Lymph node scans were matched with H&E but details were difficult to see due to low contrast.

Conclusions: This was an effort to begin delineating 3D histology and pathology of breast tissue using direct tissue imaging by OCT. Contrast and resolution are currently less than that available from glass microscopy, but potential exists for improving scan quality and for extracting more information from images (ie development of image analysis and better post-processing technique). Other specialties are now developing in-vivo microscopic tests that generate similar 3D histological images. In addition to potentially bypassing or reducing reliance on glass microscope slides, "virtual biopsy" expertise may soon be in demand by clinicians and may potentially represent a new discipline within Anatomic Pathology.

1442 The Utility of Dynamic Passive Telepathology in Neuropathology Intraoperative Consultations: A Critical Evaluation of Cytologic and Frozen Section Preparations.

M Gokden, SG Sharma, AG Saad. University of Arkansas for Medical Sciences, Little Rock; Arkansas Children's Hospital, Little Rock.

Background: Telepathology (TP) is becoming widely used especially in institutions where access to subspecialty expertise is limited. TP have been previously studied in intraoperative consultation (IOC) in neuropathology (NP), but its utility in smear preparations in NP is not well defined. We analyzed the utility of TP in NP with respect to neoplasm types, as well as the cytologic (C) and frozen section (FS) components.

Design: Total of 72 consecutive cases {43 gliomas of various histologic types and grades, 10 meningiomas, 4 lymphoid processes, 4 peripheral nerve sheath tumors (PNST), 8 metastatic carcinomas, 3 nondiagnostic cases} with available C & FS slides, were identified retrospectively from the files in 2009-2010. The TP System comprised of Nikon Digital Sight DS-L2 & Nikon Eclipse 55i microscope. It was operated by the neuropathologist (NPst) at the primary center while the NPst at the remote location reviewed the real-time images on a computer screen. Both were blinded to the final diagnoses and made independent diagnoses. The evaluation started with cytologic component and was limited initially to morphology; clinical & radiologic data were made available as requested. C & FS components were timed separately. The time used and concordance rates were analyzed; any practical issues were discussed.

Results: Average time for C/FS in seconds were: Glioma 48/69; lymphoid 54/67; metastatic carcinoma 58/49; PNST 52/109; meningioma 29/84; nondiagnostic 22/55;