Results: Compared to normal or reactive lymphoid tissue we found increased nuclear expression of localized b-catenin-pS552 in atypical lymphoid hyperplasia and extranodal marginal zone lymphoma. There was a statistically significant difference between reactive lymphoid hyperplasia, atypical lymphoid hyperplasia and marginal zone lymphoma ($P<0.05$). We followed sequentially three patients with stomach MALT lymphoma and studied their biopsies before and after treatment. The pβ-cat-S552 positive cells showed a decrease in number in response to the therapy for *H. pylori,* which also correlated with histologic remission.
Conclusions: In summary, we have demonstrated that β-cat-S552 positive nuclear stain cells were increased in number in atypical lymphoid hyperplasia and MALT lymphoma. Furthermore, we showed that these cells are located outside of germinal centers. We suggest that the antibody to β-cat-S552 can be used to assess the distribution and number of atypical lymphoid cells in the diagnosis of MALT lymphoma and for monitoring of those patients' response to therapy.

1476 Promoter Hypermethylation of *SASH1* Supports a Tumor Suppressor Role in Diffuse Large B-Cell Lymphomas

T Zhang, K Nie, R Shaknovich, DM Knowles, W Tam. Weill Cornell Medical College, New York, NY.
Background: The gene *SASH1* (SAM- and SH3-domain containing 1) has been identified as a candidate tumor suppressor gene (TSG) in breast and colon cancers. Previously, deletion mapping of chromosome 6q identified a minimal deletion region around *D6S311* in Reed-Sternberg cells of classical Hodgkin lymphoma (cHL). Sequence comparison showed that *D6S311* fell within the *SASH1* gene located in 6q24.3. Morevoer, *SASH1* is located within the frequently deleted 6q region of non-Hodgkin B-cell lymphomas. The goal of this study was to evaluate the presence of genetic and epigenetic alterations in this putative TSG in lymphomas.
Design: The entire *SASH1* coding region was sequenced in L1236, which has a 6q14.3-qter deletion. Methylation status of 94 CpG in the promoter and exon 1 of *SASH1* was assessed by bisulfite sequencing in 20 primary diffuse large B-cell lymphoma (DLBCL) clinical cases and 12 cell lines: 3 cHL (L1236, L428 and KMH2), 2 Burkitt lymphoma (Daudi, Raji), 2 germinal-center B-cell (GCB) type DLBCL (OCI-Ly1, SUDHL6), 4 non-GCB type DLBCL (OCI-Ly3, OCI-Ly10, SUDHL2 and U2932), and 1 myeloma (U266). Normal naïve B cells and GCB cells were analyzed as a control. SASH1 expressions in the cell lines, as well as normal B cells and plasma cells, were measured by quantitative RT-PCR. To determine if there is a causal relationship between promoter hypermethylation and *SASH1* transcription repression, DLBCL and cHL cell lines were treated with 5-aza-2-deoxycytidine (5-aza).
Results: No *SASH1* mutation was detected in L1236 cells. Two CG islands spanning the *SASH1* promoter and first exon were identified. While these are rarely methylated in normal B cells, they are hypermethylated in all primary DLBCL and cell lines tested. The extent of *SASH1* hypermethylation in each CpG varies from 10 to 90%. *SASH1* is expressed at low levels in naïve and GCB cells, but its expression is 5 to10 fold higher in plasma cells. All cell lines demonstrated lower *SASH1* mRNA levels relative to plasma cells. Inhibition of DNA methylation by 5-aza resulted in >100 fold increase in *SASH1* mRNA in DLBCL and Hodgkin cell lines.
Conclusions: *SASH1* is hypermethylated in primary DLBCL and B-lymphoma cell lines, resulting in its transcription inhibition. These results implicate *SASH1* as a TSG in B-cell lymphomas. In addition, its up-regulation in plasma cells suggests a role in normal B cell differentiation. Methylation analysis of additional clinical samples is ongoing to further confirm its tumor suppressor role in DLBCL and other lymphomas.

1477 Low-Level Expression of a *JAK2* Splice Variant with Exon 14 Deletion Is Common in Patients with Chronic Myeloproliferative Neoplasms

Z Zhang, W Ma, TS Lee, X Zhang, X Wang, CH Yeh, M Albitar. Quest Diagnostics Nichols Institute, San Juan Capistrano, CA; University of Minnesota, Minneapolis, MN.
Background: We previously reported detection of a *JAK2* splice variant transcript with complete deletion of exon 14 (88 bp, encompassing V617; 14-del) in rare patients with chronic myeloproliferative neoplasms (MPNs). Molecular modeling simulations suggested that 14-del will exhibit dominant-negative effects leading to constitutive activation of the JAK2-STAT pathway, similar to that caused by V617F mutation. Although 14-del was detected at levels >15% of total *JAK2* transcript (detection limit of original assay) in the previously reported cases, we speculate that some patients may express low levels of this splice variant.
Design: To determine the frequency of low-level expression, we designed a highly sensitive reverse-transcription/polymerase chain reaction (RT/PCR) test to quantify abnormally spliced 14-del transcript at levels as low as 1% of total *JAK2* transcript. This assay was used to test samples from 61 patients with confirmed MPN, 183 patients with suspected MPN, and 46 healthy normal controls.
Results: The 14-del transcript was found at low levels (2.1% to 33.9% of wild-type levels) in 60 patients: 9 (15%) with confirmed MPN, 51 (28%) with suspected MPN, and none in normal controls. Roughly one-third of V617F-negative samples (31/90) from patients with MPN or suspected MPN were positive for 14-del expression. In twenty cases (20/93), the 14-del transcript coexisted with V617F transcript.
Conclusions: Although more functional data are needed, our findings suggest that the expression of this abnormally spliced *JAK2* transcript may be a common molecular abnormality in MPN, one that may cause constitutive activation of the JAK2-STAT pathway and thus contribute to the neoplastic phenotype in MPNs.

Infections

1478 Eritematous Cutaneous Nodules in Heart-Transplanted Patients: A Sign of Chagas' Disease Reactivation

SA Araujo, RS Laboissiere, MCV Moreira, SA Andrade, AJA Barbosa. Medical School, Federal University of Minas Gerais, Belo Horizonte, Brazil.
Background: Eritematous cutaneous nodules may occur after organ transplantation due to several pathologic disorders such as graft-versus-host disease, cutaneous toxicities of drugs and numerous infectious diseases resulting mainly from bacteria, virus and fungus. Although there is limited information about its epidemiology, Chagas' disease reactivation should be one important differential diagnosis to consider in patients from Latin America, in those countries where the disease is endemic. We report here a series of five cases of heart transplanted chagasic patients who developed eritematous cutaneous nodules (skin chagoma) shortly after surgery.
Design: The five cases of chagasic patients underwent heart transplantation from 2007 to 2009 at a Brazilian University Hospital. The immunosuppression protocol was based on a combination of cyclosporin or tacrolimus with mycophenolate mofetil in addition to prednisone. All patients were on prednisone-free immunosuppression after the first 6 months following cardiac transplantation. Routine histological preparations (H&E) of skin biopsies were analyzed. The samples were also stained by immunoperoxidase technique using polyclonal rabbit antibodies against *Trypanosoma cruzi* amastigotes.
Results: The mean age of the group was 44.4 years. The patients presented their first episode of Chagas' disease reactivation, as eritematous cutaneous nodules, at a mean time of 8 weeks after surgery. One patient had a second Chagas' disease reactivation 20 months after the heart transplant. The biopsy specimen from these lesions revealed a diffuse inflammatory infiltrate, composed of lymphocytes and macrophages, presented in the upper and lower dermis, extending into the hypodermal adipose tissue (septal panniculitis). The *T. cruzi* amastigote-like microorganisms were observed in the H&E preparation and were confirmed by immunochemistry reaction. Numerous microorganisms with paranuclear kinetoplasts were seen in the cytoplasm of hystiocites, fibroblasts and endothelial cells. Some isolated amastigotes were apparently identified along the edematous interstitial space.
Conclusions: Chagas' disease reactivation in immunosuppressed patients after heart transplantation could be an important cause of cutaneous lesions. One should attempt to make this differential diagnosis when dealing with patients from endemic regions.

1479 Comparison of Molecular (Real Time PCR) Based Detection of Viruses in GI and Renal Small Biopsy Specimens to Histology Based Evaluation

K Aulakh, C Chisholm, V Zamudio, K Hocker, A Rao. Scott & White Memorial Hospital and Texas A&M Health Sciences Center, Temple, TX.
Background: Viral infections of the gastrointestinal tract and kidney can cause significant clinical disease, especially within the context of immunocompromised states including transplant patients. Some of the viruses commonly associated with GI and renal pathology include cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), adenovirus, and JC and BK viruses. Real time molecular (PCR) detection of these viruses has proven to be a sensitive and specific methodology, but there is little documentation of the increased sensitivity when compared to routine histology or in-situ hybridization. We sought to compare the techniques to evaluate sensitivity and specificity using clinical outcome.
Design: The data for this study was compiled from an institutional pathology database (2004-2009) and consisted of previously collected gastro-intestinal and kidney formalin-fixed, paraffin-embedded specimens that were tested for viruses by PCR as well histological examination.
Results: 140 patients were identified that had tissue specimens which were analyzed by real time PCR. 23 of the submitted 138 surgical specimens were positive by PCR, 18 of which were negative by histology. Eleven of these specimens were immunosuppressed patients from transplantation, acquired immunodeficiency syndrome (AIDS), or chemotherapy. No histology positive, PCR negative samples were identified. Clinical correlation was also performed and identified that all positive patients responded favorably to appropriate interventions such as antiviral therapy or temporary reduction of immunosuppression. Additionally, 4 renal biopsies were positive for BK virus. These patients had no BK viremia but were positive for BK virus in urine. Thus, clinically, molecular identification of virus in tissues may be the most relevant in determining disease.
Conclusions: Histological examination alone lacks the sensitivity and specificity when compared to PCR, particularly when classic cytological and nuclear features are absent. Early PCR testing is a sensitive and specific method to enable a more accurate tissue diagnosis, as well as faster turnaround time, thereby allowing the clinician to implement treatment more promptly. Additionally, quantitation of viral load can be performed which allows for better monitoring.

1480 ASC-US: Is There a Correlation between High-Risk HPV and Vaginal Infections

EE Bogdan, M Koch, V Du, GM Oprea-Ilies. Emory University - Oxford College, Atlanta, GA; Midwestern University, Phoenix, AZ; Emory University, Atlanta, GA.
Background: ASC-US by the new Bethesda guidelines represents a cytopathologic interpretation of uncertainty that is reflex tested for human papilloma virus (HPV) presence. FDA approved the use of HPV testing in conjunction with the liquid prep Pap Test for cervical cancer screening in March 2003. Our institution, which serves a high-risk population, uses a commercial probe to test for high-risk HPV (HR-HPV) in liquid prep Pap test.
Design: The files of our institution were searched for Pap tests diagnosed as ASC-US and HR-HPV test. These cases were studied in correlation with associated vaginal

infections: Candida (C), Bacterial Vaginosis (BV), Trichomonas vaginalis (T), Herpes virus and the amount of inflammation. Additional data included age at the time of the test. The data were entered into an excel spreadsheet. Statistical calculation was performed using chi square test.

Results: A total of 4089 Pap tests were diagnosed with ASC-US and had HR-HPV test performed. 1040 (25%) were positive and 2327 (56%) negative. 722 (19%) have not been tested for HR-HPV. The age distribution was: 3243 (80%) < 50 year of age, 846 (20%) > 50 years of age. Of the HR-HPV positive cases, 125 (12%) showed C, 99 (9.5%) showed T, 247 (23.75%) showed BV, 4 (0.38%) showed Herpes, 332 (31.9%) mild, 481 (46.25%) moderate, 153 (14.7%) severe infections and 60 (5.8%) an association of those. Of the HR-HPV negative cases, 227 (9.75%) showed Candida, 170 (7.3%) showed Trichomonas, 329 (14.1%) showed bacterial vaginosis, 2 (0.08%) showed Herpes, 724 (31.1%) mild, 1108 (47.6%) moderate, 352 (15.1%) severe infections and 135 (5.8%) an association of those. See table.

	HR-HPV +N= 1040 (25%)	HR-HPV – N=2327 (56%)	P-VALUE
CANDIDA	125 (12%)	227 (9.75%)	0.0497
TRICHOMONAS	99 (9.5%)	170 (7.3 %)	0.0369
BACTERIAL V.	247 (23.75)	329 (14.1%)	0.0001

*The other characteristics did not show significant differences.

Conclusions: Conclusions: ASC-US associated with morphologically diagnostic infectious organism is most likely to correlate with HR-HPV infection. Two explanations may be considered: 1. Women who are infected with C, T, and the organisms responsible for BV have a higher risk for other sexually transmitted diseases such as HPV. 2. A second explanation may be that cytopathologists tend to downgrade nuclear changes in the presence of overt infectious organisms.

1481 Fungal Prosthetic Valve Endocarditis: Mayo Clinic Experience Including a Clinicopathologic Analysis

JM Boland, H Chung, FJL Robberts, WR Wilson, JM Steckelberg, KL Greason, LM Baddour, DV Miller. Mayo Clinic, Rochester, MN.

Background: Fungal prosthetic valve endocarditis is a rare but devastating disease, which can lead to severe acute valve dysfunction. Data on this disease is sparse, with reports in the literature comprised largely of case reports and small series. In this study, we report an institutional experience of fungal prosthetic valve endocarditis with clinicopathologic analysis.

Design: To better characterize this rare syndrome, we retrospectively reviewed 21 cases of fungal prosthetic valve endocarditis seen at Mayo Clinic over the past 40 years.

Results: The average patient age was 65 years with a 2:1 male predominance. About half of the cases occurred within one year of prosthetic valve placement. The aortic valve was most commonly affected, and the most common etiologic organism was *Candida* species (16 cases), followed by *Histoplasma capsulatum* (3 cases). The majority of patients were immunocompetent, although they had other risk factors for fungal infection including recent cardiac surgery, antibiotic use, previous bacterial endocarditis, other systemic infections, and central venous catheters. Patients often presented with systemic signs and symptoms of infection, and cardiac imaging was abnormal in most cases. Pathological material was available on 12 of 21 cases (6 surgical specimens, 6 autopsies). Pathologic evaluation of valve material was high yield, with organisms identified in 92% of cases using H&E, GMS and/or PAS stains. The predominant histologic pattern was that of bulky fibrin and platelet-rich vegetations with scant to moderate associated mixed inflammation and necrotic debris. Some cases showed scattered giant cells, but well formed granulomas were not identified. Fungal prosthetic valve endocarditis was associated with a high morbidity and mortality, with 67% of patients experiencing complications and 57% of patients dying of infection-related disease.

Conclusions: Fungal prosthetic valve endocarditis is a rare but life threatening complication of valve replacement surgery, most commonly caused by *Candida* species. Most affected patients are immunocompetent, and can be affected any time after prosthetic valve placement. The aortic valve is most commonly involved, and vegetations are often bulky. Pathologic evaluation of valve material is high yield, with organisms identified in the vast majority of cases.

1482 Morphological and Ultrastructural Analysis of Lung Parenchyma in Patients with Swine Flu Influenza Type A/H1N1

VL Capelozzi, PRM Rocco, ER Parra, HA de Oliveira, M Ximenes, CSV Barbas, MIS Duarte. Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil; Hospital de Base do Distrito Federal, Brasilia, Brazil; Hospital Israelita Albert Einstein, São Paulo, Brazil.

Background: Severe acute respiratory failure due to Swine-Origin Influenza A H1N1 presents a mortality of 9.2%. A better understanding of the underlying mechanisms of this highly lethal new disease can lead to advances in the treatment.

Design: We describe a case series of 3 patients with confirmed diagnosis of H1N1 by positive PCR in nasal swab investigation. All of the patients had acute respiratory failure needing ventilatory support. Morphological assessment and ultrastructural study of lung tissue were performed.

Results: The pathological features were dominated by necrotizing bronchiolitis, diffuse alveolar damage and cytopathic effects. The membranous and respiratory bronchioles were widespread compromised by epithelial necrosis, squamous metaplasia and obliteration by fibroplasia. The parenchyma was modified by extensive alveolar collapse, dilatation of the airspaces, hemorrhage and hyaline membrane formation. Atypical bronchiolar and alveolar epithelial cells were seen in all patients, including multinucleated giant forms, but distinct viral inclusions were not apparent. Ultrastructural analysis showed necrosis and degenerative changes in bronchial and alveolar epithelium with sloughing of the lining cells and denudation of the basement membrane. The regenerating bronchiolar epithelium extended along adjacent alveolar septa showing features of cells with prominent surface microvilli with decreased or absent lamellar bodies and considerable cytologic atypia characterized by nuclear enlargement and clumped nuclear chromatin.The proliferating bronchiolar and alveolar epithelial cells containing tubuloreticular structures and cylindrical confronting cisternae probably representing viral-like particles residuals were distinguished in all cases.

Conclusions: Surgical lung biopsy allows tissue to be sampled for morphological and ultrastructural examination in Swine Flu Influenza A/H1N1 and showed that bronchioles and epithelium, rather than endothelium, are probably the primary target of infection and the diffuse alveolar damage the consequence of airways obliteration, suggesting that the treatment should be focused on the epithelium repair. Financial Support: FAPESP, CNPq.

1483 Cerebral Malaria as a Cause of Maternal Mortality in Endemic Areas

P Castillo, C Carrilho, MR Ismail, F Machungo, C Romagosa, A Mayor, C Menendez, J Ordi. Hospital Clinic. CRESIB, Barcelona, Spain; Hospital Central de Maputo, Maputo, Mozambique.

Background: Cerebral malaria (CM) is a frequent cause of death in children living in endemic areas. In contrast, in adulthood CM almost exclusively affects non-immune individuals living in non -or low endemic areas. Pregnant women are more susceptible to malaria than non pregnant women or men, and this is due to the presence of a subset of Plasmodium falciparum parasites that adhere to chondroitin sulphate A expressed in trophoblasts and not in other endothelial cells. Malaria in pregnancy is considered to cause severe disease (spontaneous abortion, stillbirth, and CM) only in non-immune women, whereas in semi-immune women from endemic areas, it causes only low birth weight and prematurity in the newborn and anemia in the mother but it is not considered a cause of maternal death. However, there is scant information on the possible role of malaria as cause of death in pregnant women from endemic areas. The aim of this study was to evaluate whether CM is a cause of maternal mortality in endemic areas.

Design: This was a prospective, descriptive study that included all consecutive deaths fulfilling the standard definition of the WHO for a pregnancy-related death in a tertiary level referral hospital in Maputo, Mozambique, between October 2002 and December 2006. A complete dissection with macroscopic evaluation of each organ was performed by a pathologist using a standardized macroscopic protocol. Samples of all grossly identified lesions and of all viscera were collected in each case for histological study.

Results: During the study period, there were 316 complete autopsies of pregnancy-related deaths. CM was identified in 10 cases (3.2%). In all cases massive accumulation of sequestered parasitized erythrocytes were identified in small capillaries in the central nervous system. In all cases most peripheral capillaries from the viscera showed abundant sequestered parasitized erythrocytes. Placenta, which was available in 4 cases showed a massive accumulation of parasites in the maternal erythrocytes in the intervillous space in all cases.

Conclusions: (1) Cerebral malaria is an infrequent (3.2%) but definite cause of maternal mortality in endemic areas; (2) phenotypes of P. falciparum adherent to trophoblast and cerebral capillaries coexist in these patients. Work support in part by grant PI060207 from the Fondo de Investigaciones Sanitarias.

1484 Molecular Analysis of *Clostridium difficile*, C. difficile 027/B1/NAP1 and Vancomycin-Resistant Enterococci (VRE) in Tissues with Pseudomembranous Colitis or CDAD

C Chisholm, D Smith, G Frederick, K Hocker, A Rao. Scott & White Memorial Hospital and Texas A&M Health Sciences Center, Temple, TX; University of Mary Hardin-Baylor, Belton, TX.

Background: CDAD (Clostridium difficile associated colitis) can present with disease ranging from diarrhea to severe pseudomembranous colitis (PMC). The identification of a unique strain, 027/B1/NAP-1, with a mutation in the negative regulator tcdC gene, is associated with increased virulence. We investigated the prevalence of C. difficile with toxin A and B genes, the 027/B1/NAP1 strain and the presence of VRE organisms in biopsies submitted from CDAD symptomatic patients.

Design: 90 paraffin-embedded surgical pathology specimens associated with patients with clinical pseudomembranous colitis or CDAD were analyzed for the presence of VRE and *C. difficile* by PCR identifying toxin A and B genes. A separate PCR analysis identified the 027/BI/NAP1 mutation. All corresponding *C. difficile* toxin EIA and stool PCR results were collated from the records.

Results: 45 tissue samples had PMC in the histological diagnosis. 25 of these had molecular evidence of VRE or *C. difficile* and stool PCR, toxin EIA, and tissue PCR were positive in 86%, 60%, and 38% of these patients with both toxins A and B genes identified. Only 1 patient was positive for the 027/B1/NAP1 mutation. Interestingly, in 7 of the 45 PMC patients, VRE alone was detected both in tissue and stool. 8 patients had both *C. difficile* and VRE in tissue and/or stool. When both organisms were identified, the average white blood cell count was 37 as compared to those with only VRE or *C. difficile* (24.3 and 22.0). Worse renal function and increased morbidity was correlated with VRE. 45 additional samples had a clinical diagnosis of CDAD but had no histological changes of PMC. Only 3 of these were positive by molecular analysis for C. difficile and/or VRE.

Conclusions: *C. difficile* 027/B1/NAP1 was seen in only 1 case, while 2 patients had an alternate tcdC allele B. Thus, VRE emerged as a major contributor to morbidity and mortality in patients with PMC even when compared to the virulent C. difficile strains. It is likely an under-recognized causal agent and it may be critical to examine patients for VRE.

1485 A Series of Patients with Primary Central Nervous System Lymphoma (PCNSL) and AIDS

JE Eisenstein, MB Mosunjac. Emory University School of Medicine, Atlanta, GA; Grady Memorial Hospital, Atlanta, GA.

Background: Distinguishing toxoplasmosis from primary central nervous system lymphoma (PCNSL) radiologically in patients with AIDS is difficult. The gold standard for making the diagnosis of PCNSL is stereotactic biopsy with histological evaluation.

Design: A data search was performed at Grady Memorial Hospital's pathology records from 1989-2009 for biopsy proven PCNSL diagnoses. AIDS status, clinical presentation, and radiologic findings were correlated using laboratory and medical records.

Results: 25 cases of biopsy/autopsy proven PCSNL were identified. 22 of those patients had AIDS at the time of diagnosis. Mean patient age at the time of diagnosis was 38.9 yrs (range: 26-63 yrs). Of the 22 patients, 8 presented initially with altered mental status, 5 with motor symptoms including weakness and numbness, 5 with general malaise, and 4 with seizures. Brain imaging revealed multiple enhancing lesions in 12 patients, single enhancing lesions in 8 patients, and no documented historical imaging in 2 patients. Of the 22 patients with AIDS, within a year of their diagnosis of PCNSL, patients' CD4 counts averaged 17 (range: 1-88). Fourteen (64%) of these patients were documented to have been prescribed antiretroviral therapy. Of those on antiretrovirals the mean time of HIV/AIDS diagnosis to the diagnosis of PCNSL was 53.1 months (range: 6 – 168 months, SD = 50.66) compared to patients not documented to be on antiretrovirals [18.8 months (range: 1 – 60 months, SD=25.19)]. Death dates were documented in 9 patients, 3 of which had PCNSL histologically diagnosed at autopsy (1992 - 2005). Patients had a mean lifespan from diagnosis of HIV/AIDS of 32.3 months (range 2-84 months) and from diagnosis of PCNSL of 1.2 months (range: 0-5 months).

Conclusions: PCNSL at our institution over a 20 year period is a rare biopsy proven diagnosis, yet is highly associated with AIDS (22 of 25 cases). The presumptive diagnosis of PCNSL becomes main differential diagnosis when treatment for toxoplasmosis cases fails to show clinical and radiologic improvement. The dismal prognosis in our patients after the diagnoses of PCNSL is made is apparent, yet unknown death dates on those patients who received whole brain irradiation from 2006 – 2009 requires further investigation.

1486 Morphological and Ultra Structural Findings in Influenza A (H1N1)

A Fernandez, K Arispe, D Moran, JC Leon, D Montante, E Reyes-Gutierrez, A Angeles-Angeles, A Gamboa-Dominguez. Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Tlalpan, Mexico.

Background: In March an outbreak of severe respiratory infection began in Mexico that was subsequently attributed to a novel human/swine origin influenza A (H1N1) virus. A second wave of illness started in September 2009.

Design: Objective: to describe the demographic, clinical and morphological findings in surgical and autopsies studied in a referral center in Mexico City. Material and methods: Autopsies and open lung biopsies of suspected cases of influenza A (H1N1) were included in this series. Information of nasopharyngeal swab tested for nucleic acid detection was searched in charts. The demographic, clinical course, co morbidities, morphological and ultra structural characteristics of positive cases is described.

Results: Since March four autopsies were performed out of five deceased positive patients, and an open lung biopsy of an external patient. All cases started one to five days previous to develop acute respiratory distress and to require mechanical ventilator support. Age ranged from 32 to 56 years with a mean of 41. Initial complaints were productive cough, fever, chest pain and dyspnea in all cases. Co morbidities were documented in all death patients (constrictive pericarditis, chronic malnourishment, systemic lupus erythematosus and anorexia), but in the biopsied patient who was the only male and previously in good health. In 2/5 patients oseltamivir was started without clinical improvement. Anemia and peripheral cytopenia were documented in autopsied cases. Hydrothorax, hemorrhagic lung consolidation and diffuse alveolar damage were documented in all patients. Lung, lymph node, bone marrow and liver hemophagocytosis was observed. Alveolar macrophages showed electron dense structures surrounded by membranes close to nuclear envelope and to the cytoplasmic membrane.

Conclusions: Influenza A (H1N1) complicate the clinical course of chronically ill young patients and is associated with diffuse alveolar damage, hemophagocytosis and electron dense structures in alveolar macrophages.

1487 The Pivotal Role of Histopathology in the Diagnosis of a Novel Old World Arenavirus Viral Haemorrhagic Fever Outbreak in Southern Africa

MJ Hale, SR Zaki, CD Paddock, W-J Shieh, JT Paweska, R Swanepoel. University of the Witwatersrand & National Health Laboratory Service, Johannesburg, South Africa; Centers for Disease Control and Prevention, Atlanta, GA; National Institute for Communicable Disease, Johannesburg, South Africa.

Background: Viral haemorrhagic fevers are caused by a spectrum of viruses including arenaviruses and are endemic to Southern Africa with a high index of suspicion in the minds of medical staff attending to patients presenting with fever, a bleeding diathesis, petechial rash and altered consciousness. Such patients are investigated for a trio of infections including meningococcal meningitis, tick bite fever and viral haemorrhagic fever. Serology and culture are pivotal to confirmation of the diagnosis and initiation of containment measures.

Design: The index patient, an adult female, was evacuated by air from Zambia to South Africa with severe myalgia, facial swelling, sore throat, a petechial rash and liver failure. Three days after admission she died. Nine days after exposure, a paramedic who attended the index patient on the flight became ill with similar symptoms and died 12 days later. A further 3 patients with either secondary or tertiary contact became ill, 2 of whom died with the third surviving after protracted care. A haemorrhagic fever outbreak was considered, but as repeated serology and RT-PCR for known viruses was negative it was decided that post-mortem liver and skin biopsy were essential in the two patients in whom this was possible. These biopsies, performed under P4 conditions were retained for histology, electron microscopy and viral culture.

Results: Histology of the liver and skin biopsies showed features of a haemorrhagic fever. Extensive pan lobular hepatocellular necrosis was identified in both patients, with numerous acidophil bodies, severe intracellular cholestasis, macrovesicular steatosis and lymphocytic infiltration. Immunohistochemistry for Old World arenavirus antigen showed cytoplasmic staining confirming the H&E diagnosis of a viral haemorrhagic fever, providing the first evidence of a potential aetiology. The skin biopsies showed a lymphocytic vasculitis of dermal blood vessels with infarction of the overlying epidermis. Later, molecular methodology and viral culture identified the virus as a novel highly pathogenic arenavirus now named the Lujo virus.

Conclusions: This report demonstrates the vital role of histology in the diagnosis of hitherto undescribed disease.

1488 Utilization of Single Nucleotide Polymorphisms To Detect and Speciate Atypical Mycobacteria in Formalin Fixed, Paraffin Embedded Specimens

JM Havens, LJ Gilbrech, SA Schichman, LW Lamps. Univ. Arkansas for Medical Sciences, Little Rock, AR; Central Arkansas Veterans Healthcare System, Little Rock, AR.

Background: Non-tubucular mycobacterial (MOTT) infections are increasingly recognized as important causes of pneumonia and extrapulmonary infections, particularly in immunocompromised patients. Distinction of MOTT from *M. tuberculosis*, as well as identification of the MOTT species, is important because drug therapy differs. Diagnosis often requires time-consuming cultures and/or PCR and sequencing. Furthermore, many assays cannot be used in formalin fixed, paraffin-embedded (FFPE) tissues. Our goal was to develop a molecular assay that detects and distinguishes between 12 species of MOTT (including *M. avium-intracellularae [MAI]; M. gordonae [MG]; M. simiae [MSi]; M. kansasii; M. malmiennse [MMa]; M. gastri [MGa]; M. marinum; M. scrofulaceum [MSc]; M. asiaticum; M. szulgai;* and *M. leprae*), for use in FFPE tissues.

Design: Novel primers were designed exploiting single nucleotide polymorphisms (SNPs), which allows for subtle distinction between genetically similar species that cannot be detected by conventional PCR. The primers were used in conjunction with the ABI SNaPshot kit, which is designed for use with multiplex assays. 27 known MOTT positive FFPE specimens (including skin, lung, and intestine) were analyzed. Following deparaffinization and DNA extraction, a 130-bp fragment of the IST1 interspace region was amplified by PCR. The amplicons were then subjected to a second multiplex reaction using the novel primers. Capillary electrophoresis using an ABI 3100 genetic analyzer was used for detection. Known culture strains obtained from ATCC and known positive cases served as positive controls. Reagent blanks served as negative controls.

Results: 23 of 27 cases contained MOTT DNA by PCR; 4 cases did not contain amplifiable DNA. Species was determined in all 23 amplified cases as well (7 MAI, 3 MG, 4 MGa, 3 MSi, 2 MMa, 4 MSc). All positive and negative controls worked appropriately.

Conclusions: Using our novel assay, the MOTT species was determined in all 23 cases containing amplifiable DNA. Since the diagnosis of MOTT was considered retrospectively in these cases, culture was not performed, and thus comparison of this assay to other methods (including culture, AFB stains, and conventional PCR/sequencing) requires further study. However, our preliminary data indicate that the use of single nucleotide polymorphisms is an excellent method for detection and speciation of MOTT in FFPE tissues.

1489 Anogenital Herpes Simplex Virus Pseudotumor: An Unusual Variant of HSV Infection Simulating Squamous Cell Carcinoma in HIV Patients

EF Krasik, JT Rabban. University of California at San Francisco, San Francisco, CA.

Background: Anogenital herpes simplex virus (HSV) infection typically manifests as a shallow ulcer on vulvar, perineal, penile or scrotal skin. Rare cases of tumor-like presentations of HSV in various organs exist. We report a series of anogenital HSV pseudotumors in HIV patients with mass-like features simulating squamous cell carcinoma, emphasizing potential morphologic diagnostic pitfalls.

Design: All anogenital surgical specimens with a diagnosis of HSV were identified from our general surgical pathology files between 1989 and 2009. Clinical and laboratory data were obtained from the patients' charts. Cases were classified as a mass-like lesion or not based on their clinical exam findings; slides and pathology reports were reviewed.

Results: Among 49 surgical specimens of anogenital HSV, 6 patients presented with tumor-like growths (2.8 cm to 10 cm). All 6 were HIV patients (2 female, 4 male; age 43 to 53 years). Pseudotumors arose in the perianal region (4), vulva (1), or scrotum (1). Pre-biopsy clinical suspicion was neoplasm (2), infection (2) or unreported (2). Procedures included local excision (4), wide excision (1), or biopsy (1). Grossly, pseudotumors consisted of bulky, exophytic growths with variable surface erosion. Microscopically, two morphologic zones were noted: A) a superficial layer of pseudocarcinomatous epithelial hyperplasia with an underlying zone of dense lymphoplasmacytic inflammation, exuberant granulation tissue and sparse viral infected cells and B) a deeper dermal layer of dense, well-organized fibrotic tissue containing lymphoid aggregates. A variable degree of ulceration and acute inflammation involved the epidermis. HSV-infected cells were present mostly at the dermal-epidermal junction and their number and distribution was sparse, even with HSV immunohistochemistry. Some tissue sections did not contain any infected cells. Co-infection with cytomegalovirus or fungi was not seen. One case was originally interpreted to be squamous cell carcinoma, but only 1 case contained focal severe squamous dysplasia. Average follow up was 41 months (range 0 to 95 months). Recurrent pseudotumor lesions arose in 4/6 patients.

Conclusions: Anogenital pseudotumor is an unusual form of HSV infection in HIV patients that may pose diagnostic piftalls. Pseudocarcinomatous epithelial hyperplasia can mimic squamous carcinoma in shallow biopsies. Viral infected cells may be sparse, requiring thorough sampling of the mass. Co-existence of squamous dysplasia should also be considered when sampling.

1490 Comparison of Detection Methods for the Rapid Identification of Toxigenic *Clostridium difficile*

ON Kryvenko, LP Samuel, RJ Tibbetts. Henry Ford Hospital, Detroit, MI.

Background: *Clostridium difficile* associated disease (CDAD) is a common nosocomial complication resulting in hospital-associated diarrhea following administration of broad-spectrum antibiotics. In spite of the growing incidence of CDAD the sensitivity of the enzyme immunoassay (EIA), the most commonly used diagnostic test, remains suboptimal. Our goal was to increase sensitivity and specificity while preserving the short turnaround time and relatively low cost of the diagnostic algorithm.

Design: We conducted a prospective study in which we analyzed 294 consecutively submitted specimens for *C. diff* by EIA, antigen card assay (AA), antigen/toxin combination card assay (ATA), and PCR. Discrepant results were sent out for toxigenic culture, currently the gold standard for *C. diff* detection.

Results: The prevalence of CDAD in tested population was 9.5%. EIA in our hands had a sensitivity ~68%, specificity ~98%, PPV of 76%, and NPV of 96.65%, which is in keeping with reports in the literature. The EIA had a false positive rate of 2.04% (6/294) and false negative of 3.06% (9/294) with resultant patient health risk and related medicolegal and financial issues. AA had a NPV of 100% but specificity of 82.71%. ATA combination had a PPV of 100% and a sensitivity of 64.29%. The PCR had sensitivity of 85.71% and specificity of 96.60%. The PCR testing of all ATA assay antigen positive and toxin negative results (11.22% of tested individuals) identified all cases that were falsely toxin negative. This combination of ATA and PCR achieved sensitivity of 100%, specificity of 98.87%, PPV of 90.32%, and NPV of 100%. The overall cost is 2.75 times higher than that of EIA along which is negligible compared to expenses related to wrong results.

Conclusions: The combination of ATA and PCR is a powerful diagnostic tool for screening and confirmation of CDAD. The antigen component of ATA with a NPV of 100% reliably screens out healthy individuals. The ATA has NPV of 96.38% and PPV of 100% which markedly reduces the number of confirmatory PCR tests. ATA is a rapid manual test performed without sophisticated equipment, it has a random access nature and a set of 30 tests will need 45 minutes to be completed in experienced hands. Addition of confirmatory PCR to antigen/toxin combination in cases of discrepant results allows maintaining NPV at 100% level with very high specificity while having roughly 1% of falsely positive results. The application of this combination of tests is a very cost effective diagnostic tool with dramatic improvement in patient care.

1491 Morphology and Immunophenotype of Infectious Mononucleosis Presenting in Axillary and Inguinal Lymph Nodes and Other Unusual Locations

A Louissaint, JA Ferry, NL Harris, LR Zukerberg. Massachusetts General Hospital, Boston, MA.

Background: Infectious mononucleosis (IM) due to Epstein-Barr virus (EBV) mainly affects adolescents and young adults, and often results in enlargement of cervical lymph nodes (LN) and Waldeyer ring (WR) tissue.The diagnosis is usually made based on clinical and laboratory findings. Occasionally, IM can present with an enlarged LN or lymphoid mass outside of the cervical and WR region. Such atypical presentations occur most commonly in older patients and a misdiagnosis of lymphoma is not uncommon on biopsy. The morphological and immunophenotypic features of lymphoid tissues in these unusual sites have not been well described.

Design: 10 cases of IM involving inguinal lymph nodes and other unusual sites were identified. Histologic slides were reviewed, immunohistochemistry (IHC) performed, and clinical data obtained from patient records.

Results: Specimens included inguinal LNs (6), axillary LNs (2), conjunctiva (1) and large bowel (1). There were 5 M and 5 F, aged 11 -88 years (median 43). Patients had fever (4/10), splenomegaly (3/10) and/or generalized lymphadenopathy (5/10). Most patients (8/10) had a positive EBV serology. No patient developed lymphoma (f/u 2-96 months [median 36]). LN and mucosal tissues showed architectural distortion by a polymorphous atypical lymphoid infiltrate, often associated with vascular proliferation and a predominance of CD3+ T cells. These features often suggested AITL (4/10). Other cases had bizarre immunoblasts mimicking R-S cells (5/10) that were CD30+, OCT2+ and Bob.1+, but CD15-. The majority of immunoblasts were CD 20+, but in some cases were CD3+CD8+ T cells. Immunoblasts were CD30+(6/6), CD15-(6/6), MUM1+ (3/3), BCL6- (6/6), and CD10- (6/6). BCL2 was variably expressed. Unlike cases of IM in cervical LN/WR, necrosis was not a significant feature (3/10). PCR analysis for T cell rearrangements revealed a small clonal population in 3/10 cases. EBER was positive in all cases.

Conclusions: IM involving extranodal sites other than WR is associated with architectural distortion by an atypical lymphoid infiltrate that may be worrisome for lymphoma on biopsy. IM should be considered in all immunoblastic proliferations - even those occurring in older patients and/or sites outside of the head / neck region. The EBV+ B immunoblasts in IM express MUM1, Oct2, Bob.1, CD30+ and lack CD15, BCL6 and CD10. This expression profile in atypical cells is unusual in DLBCL, T cell lymphoma, and Hodgkins lymphoma, and thus should suggest IM.

1492 Pathology of Placental Malaria in Areas of Different Endemicity: A Histologic Grading Scheme

A Muehlenbachs, M Fried, R McGready, TK Mutabingwa, CL Fligner, F Nosten, PE Duffy. University of Washington, Seattle, WA; Seattle Biomedical Research Institute, Seattle; Shoklo Malaria Research Unit, Mae Sot, Thailand; National Institute of Medical Research, Dar Es Salaam, Tanzania, United Republic of.

Background: During placental malaria (PM), Plasmodium falciparum infected erythrocytes sequester in the placenta and cause an inflammatory response that is harmful to the fetus and the mother. The clinical and epidemiologic features of PM differ by transmission intensity. Placental malaria episodes have been histologically classified as acute or chronic. However chronic placental malaria is a broad category that encompasses several distinct features of infection, specifically placental inflammation and malarial pigment deposition.

Design: Using frozen section histology from placentas from Tanzania, we describe a pathologic grading and staging scheme to evaluate inflammation and pigment deposition during placental malaria at delivery (n=91). For comparison, samples from placentas from Karen women on the Thai-Burma border were selected from the small fraction of women known to have had P. falciparum at least 10 days prior to delivery (n=19).

Results: In the Tanzanian cohort, the placental inflammation grade was associated with levels of inflammatory markers at delivery, and the pigment deposition stage was independently associated with birth weight. In the cohort from Thailand, inflammation and pigment deposition were associated with birth weight, and pigment deposition had an inverse trend with the number of antenatal clinic visits. The samples from Thailand had decreased pigment deposition, but similar inflammation scores when compared to samples from Tanzanian first time mothers.

Conclusions: The proposed pathological grading system is simple yet captures increased complexity of PM episodes, suggesting that combining these two measures can improve immunocorrelation for clinical trials across areas of differing endemicity.

1493 Pathologic Studies of Fatal 2009 Pandemic Influenza A (H1N1) Virus Infection in the U.S.

WJ Shieh, DM Blau, P Adem, L Liu, A Schmitz, AM Denison, J Bhatnagar, M Deleon-Carnes, J Sumner, HA Jost, P Greer, C Smith, B Batten, T Jones, C Seales, L White, J Montague, J Bartlett, C Goldsmith, D Rollin, M Patel, SR Zaki. Centers for Disease Control and Prevention, Atlanta, GA.

Background: On June 11, 2009, the World Health Organization declared the current circulating respiratory disease as an influenza pandemic, the first one in over 40 years. During the first four months after identification of a novel influenza A (H1N1) virus in the United States, over 500 deaths had been associated with infection by this virus. Pathologic studies on a series of fatal cases during that time are described in this report.

Design: Autopsy tissue samples with available clinical history and epidemiologic information were obtained from 77 patients with confirmed 2009 pandemic influenza A (H1N1) virus infection. The specimens were evaluated by an array of laboratory methods, including histopathologic examination, special stains, immunohistochemical assays (IHC), electron microscopy, and molecular techniques.

Results: Of the 77 cases, 36 (47%) were confirmed from specimens obtained at postmortem examination. The age range of the patients in this study was 2 months to 84 years with a median age of 39 years, and 81% between ages 20 to 60 years. Ninety percent of the cases included in this study had at least one underlying medical condition. Obesity (49%), cardiovascular disease/hypertension (30%) and asthma (24%) were the three most frequent pre-existing conditions in these patients. The most prominent histopathologic features were various degrees of diffuse alveolar damage. Type II pneumocytes and alveolar lining cells were the main targets involved in the infection. Thin-sectioned electron microscopy demonstrated extracellular viral particles in the alveolar space, associated with dense material. Co-infections with a variety of bacterial organisms were identified in almost one-third of the cases.

Conclusions: Our studies underscore the importance of performing autopsies and testing postmortem tissue samples in investigating 2009 pandemic influenza A (H1N1) virus infection. The most prominent histopathologic feature in fatal cases is various degrees of diffuse alveolar damage. RT-PCR and IHC assays are instrumental in establishing diagnosis and studying pathogenesis of this novel influenza virus infection. In addition, a combination of pathologic methods provides valuable diagnostic information to identify etiologic bacterial organisms as the source of co-infection.

1494 Extraintestinal Hepatobiliary Infection by *Isospora belli* in an Immunocompetent Patient: Report of First and Second Case and Review of Literature

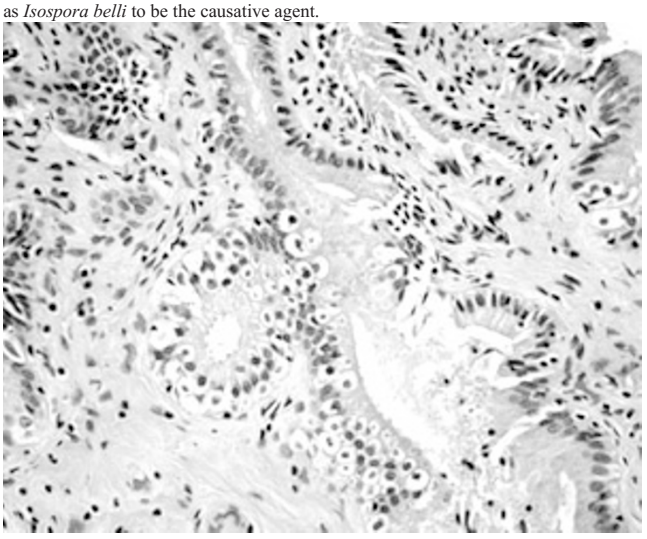
S Yadrandji. Montgomery Regional Hospital, Blacksburg, VA.

Background: *Isospora belli*, a Coccidian sporozoan, is a rare but a known intracellular parasitic infection of the human intestine causing mild to self-limited diarrhea. Extraintestinal hepatobiliary infections by *Isospora belli* are rare but reported in immunodeficient patients especially those with acquired immunodeficiency syndrome (AIDS) presenting with significant morbidity.

Design: We identified two cases of hepatobiliary infection by *Isospora belli* in our pathology department in a span of 16 months. Clinical features, radiologic studies, gross reports and histologic slides were reviewed.

Results: The two cases include one man and one woman ranging in age from 19 to 52 years old. Both parents had no history of immunosupression or previous hepatobiliary disease. The gross analysis of gall bladder showed marked edema throughout the wall however there were no stones present. The gall bladder ejection fraction in both patients was decreased and in one patient was almost zero. Microscopic examination of the gallbladder showed mild focally chronic inflammatory infiltrates. The lining epithelial cells were remarkable for presence of numerous intracytoplsmic inclusions, consistent with parasitophorous vacuoles. Numerous schizonts and macrogametocytes indicating

sexual and asexual forms were identified which interestingly were PAS positive. The liver biopsy in one patient showed non-caseating microscopic granulomata and the PAS stain was negative. The first case was diagnosed as parasitic infection consistent with cryptosporidium and were sent for second opinion and further classification of organism to a major outside institution and the report identified the coccidial parasite as *Isospora belli* to be the causative agent.



Conclusions: Significant extraintestinal complications especially cholecystitis in immunocompromised patients have been reported however to the best of our knowledge the hepatobiliary isosporiasis is not yet documented in immunocompetent patients. This report is the first and second case of isosporiasis of hepatobiliary infection in immunocompetent patients. These findings are reminders to consider the possibility of *Isospora belli* in immunocompetent patients presenting with somewhat atypical symptoms.

1495 TLR4 in Facilitating HSV-1 Neuronal Spread in Experimental Acute Retinal Necrosis (ARN) Model

M Zheng, MA Fields, Y Liu, HM Cathcart, SS Atherton. Medical College of Georgia, Augusta, GA.

Background: ARN is a rare disease usually caused by neurotropic human HSV-1. TLR4 is an innate immune mediator against a variety of pathogens, especially bacteria. Recent studies pointed toward TLR4 playing a role in a growing list of virus induced diseases.

Design: Both TLR4 mutant (mu) and wild type (wt) mice were infected with HSV-1 via anterior chamber (AC) or intravitreal inoculation. At different times post infection (p.i.), mice were sacrificed and the inoculated eyes, visual pathway containing optic nerve, chiasm, tract, and brain tissues containing the superior colliculus (SC) and lateral geniculate nucleus (LGN) were isolated. Immunohistochemistry (IHC), RT PCR, flow cytometry (FC) and virus titration were performed on the samples.

Results: Our previous results showed that after HSV-1 AC inoculation, few uninoculated, contralateral eyes of mu mice developed ARN, while ARN was observed in the majority of uninoculated eyes of wt mice. Further study revealed that an increase in certain cytokine responses at day 3 and 7 p.i. in the HSV-1 injected eye of mu compared with wt mice. FC showed more cells were infected by HSV-1 in mu compared with wt mice in virus inoculated ocular cells at day 4 p.i. One step growth curve in retinal pigment epithelial cells isolated from either mu or wt mice showed that the replication kinetics of HSV-1 were similar in both. To further investigate the role of TLR4 in virus propagation in neuronal cells within the visual pathway, HSV-1 was injected intravitreally. IHC demonstrated that HSV-1 had infected the retinal ganglion cells, then the inner nuclear cell layer cells in wt mice, whereas in mu mice, fewer cells of the subjacent retina were infected at day 2 p.i. Most HSV-1 + cells showed distinctive beta III-tubulin staining. The optic nerve was HSV-1 + at day 2 p.i. in wt mice whereas in mu mice, at day 4 p.i. The arrival of virus in the optic chiasm, tract, SC, and LGN was similarly delayed in mu compared with wt mice. IHC revealed that TLR4 was in close association with beta III-tubulin in HSV-1 infected rat retinal ganglion cells.

Conclusions: Although absence of TLR4 leads more cells to be infected by HSV-1 in virus inoculated AC, lack/delay of neuronal spread after AC or intravitreal injection of HSV-1 in mu mice suggests that TLR4 may play a role in facilitating HSV-1 transport along microtubules within the visual pathway.

Informatics

1496 Development and Use of Genitourinary Pathology Digital Teaching Set for Trainee Education and Quality Assurance

L Li, BJ Dangott, AV Parwani. Albany Medical Center, Albany, NY; University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Automated, high-speed, high-resolution whole slide imaging (WSI) robots are becoming increasingly robust and capable. This technology has started to have a significant impact on pathology practice in various aspects including resident education. Training in pathology is dependent on gaining broad exposure to these diagnostic patterns through teaching sets composed of glass slides. Whole slide imaging can provide additional educational benefits to using glass sides.

Design: A teaching set of over 250 glass slides has been used for resident education at the Division of Genitourinary Pathology, Department of Pathology, University of Pittsburgh Medical Center. Whole slide images are prepared using Aperio ScanScope CS scanner from these slides, which are de-identified. A web-based digital teaching model has been implemented at our institute using Oracle11g as the database server, SunOne as the web server, ColdFusion as the programming language, and a web middleware program to dynamically display information from a database. Case related information was obtained from electronic pathologic reports and uploaded with the corresponding whole slide images to the teaching model via a web-based data entry tool.

Results: The web site is available at: https://secure.opi.upmc.edu/genitourinary/index.cfm . It requires registration and log in. Once logged in, users can view the list of cases, and choose to show or hide the diagnoses. The search function allows searching by diagnosis or ICD-O site. A radio button is associated with each case which enables access to the case. ICD-O site, clinical history and gross description are initially shown. Whole slide images can be accessed by the links on the page that allows user to make diagnoses on their own. More information including final diagnosis will display when the diagnosis-button is clicked.

Conclusions: The web-based digital study set allows remote access to whole slide images and related information at the user's convenience. Searching and sorting functions and self-testing mode can be built in allowing more targeted study. The digital images can be annotated and the annotation can be displayed or hidden. Further, the model can be expanded to include pre-rotation and post-rotation exams, and/or to a virtual rotation system, which may potentially make standardization of pathology resident teaching possible in the future.

1497 Detection and Classification of Thyroid Follicular Lesions Based on Nuclear Structure from Histopathology Images

JA Ozolek, W Wang, GK Rohde. University of Pittsburgh, Pittsburgh, PA; Carnegie Mellon University, Pittsburgh, PA.

Background: Follicular adenoma (FA) and follicular carcinoma (FTC) are tedious challenges in surgical pathology due to lack of discriminatory cytological and microarchitectural features. Limitations of the diagnostic algorithm include time consuming tissue processing and microscopic evaluation. The aim was to develop an automated image analysis technique that could classify these lesions with 100% accuracy from routinely processed tissue using nuclear structure.

Design: Cases included 5 FA and 5 FTC resections. Sections were stained using Feulgen technique. Nuclei were segmented using random field graph cut and efficient level set active contour algorithms to yield 871 NL, 489 FA, and 703 FTC nuclei. 125 features were extracted from each nucleus. Four different classifiers (Mahalanobis distance nearest neighbors and support vector machine with different kernels) and voting strategy were used. Unique chromatin patterns were identified in feature space by finding nuclei near to each other and most distant from nuclei in other classes.

Results: These methods automatically classify the data with 100% accuracy after blind cross validation using at most 43 nuclei randomly selected from each patient.

Table 1: Classifying individual human cases using a leave one out cross validation strategy

	NL	FA	FTC
NL	10	0	0
FA	0	5	0
FTC	0	0	5

Two-dimensional representation of nuclear population with axes corresponding to directions computed by multi-dimensional scaling technique (MDS) are shown in Figure 1A (below).