reactive nodes and non-Hodgkin B-cell lymphomas. Furthermore, compared to reactive lymph nodes, cases of HL were more likely to have CD4/CD8 ratios ≥ 5.0 (15/24 vs. 10/44, P = 0.0032, Fisher's Exact). This cut-off value for CD4/CD8 ratio was determined to provide the highest sensitivity (62.5%) and highest specificity (78.6%) as compared to cut-off values of 6.0, 7.0, 8.0, 9.0 or 10.0. In addition, CD4/CD8 ratios of greater than 10 were observed in 3 cases (12.5%) of HL and 1 case (2.3%) of reactive node. None of the cases showed deletion of CD7 in T cell populations.

Conclusions: Although flow cytometry cannot provide direct diangostic evidence for HL, quantitative analysis of CD4/CD8 ratio by flow cytometry may be a useful adjunct tool in the diagnosis of HL. An elevated CD4/CD8 ratio of ≥5 should alert the hematopathologist or referring general pathologist to evaluate the lymph node carefully for histologic evidence of HL.

1201 Diagnostic Utility of Mast Cell Tryptase Immunostaining in Bone Marrow Core Biopsies in Myelodysplastic Syndromes (MDS) and Chronic Myelomonocytic Leukemia (CMML)

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Background: Patients with MDS and CMML have been shown to have increased mast cells (MC) by morphologic evaluation of marrow aspirate smears. However, when marrow aspirates are not available, it is difficult to recognize MC by morphologic evaluation of H&E stained marrow core biopsy. The aim of this study was to evaluate the expression pattern of mast cell tryptase (MTC) in the bone marrow core biopsies of MDS and CMML patients by immunohistochemistry (IHC), investigating its value in the diagnosis of these conditions.

Design: Expression of MCT was analyzed by IHC in the bone marrow core biopsies from 18 MDS patients [1 RA; 4 RARS; 8 RAEB; and 5 RAEB-T (AML by WHO classification)] and 4 CMML cases. Nine cases with marrow core biopsies showing hypercellular marrow for age and free of hematologic malignancies were used as controls.

Results: In all cases, mast cells were highlighted by strong cytoplasmic staining with minimal nonspecific staining, resulting in easy interpretation and detection of mast cells . The percentage of mast cells were as follows: 9 control cases: <1% (7) and 1-2% (2); 18 MDS cases: <1% (4), 1-2.9% (8), 3-4.9% (2), and 5-8% (4); 4 CMML cases: <1% (2), and 5-8% (2). The cases with MDS or CMML were more likely to have >1% mast cells compared to the controls (16/22 vs 2/9, p = 0.0167, Fisher's Exact). Furthermore, none of the low-grade MDS cases (RA, RARS) showed ≥3% mast cells; while, 6 high-grade MDS cases (RAEB, RAEB-T/AML) showed ≥3% mast cells (0/5 vs 6/13, p = 0.11, Fisher's Exact). The percentages of the cases with MDS or CMML showing cytogenetic abnormalities were as follows: cases with <1% mast cells: 57%; cases with ≥3% mast cells: 72%; and cases with ≥5% mast cells: 100%.

Conclusions: Mast cell tryptase immunostaining in bone marrow core biopies can be readily interpreted. Increased mast cells (>1%) highlighted by mast cell tryptase staining in core biopsies are frequently observed in patients wtih MDS or CMML. Additionally, patients with high-grade MDS tend to have even higher degree of increase in mast cells (≥3%). These findings suggest that mast cell tryptase immunostaining may be useful as an adjunct tool in establishing the diagnosis of MDS or CMML.

1202 Utility of Skp-2 and MIB-1 in Grading Follicular Lymphomas Using Quantitative Image Analysis

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Background: Follicular lymphoma (FL) is classified into grades 1, 2 and 3, based on counting the number of centroblasts in neoplastic follicles. Certain cells (follicular dendritic cells and histiocytes) may mimic centroblast morphology, limiting the accuracy of manual counts. The reproducibility of grading of FL is dependent upon observer experience; significant variations occur. A few recent studies have shown correlation between the proliferation markers MIB-1 (Ki-67) and Skp-2 among different grades of FL using manual counting. To explore a more objective, reliable way of grading FL, we have used a quantitative image analysis system in conjunction with immunohistochemical staining using antibodies to MIB-1 and Skp-2.

Design: Forty-five FL were in the study (grade [G]1, n=21; G2, n=7 and G3, n=17). Immunohistochemical stains on formalin fixed, paraffin- embedded sections used monoclonal anti-Ki-67 and polyclonal anti-Skp2 antibodies were performed with an Envision-HRP kit. Positive nuclear staining of both Ki-67 and Skp2 was recorded using the quantitative Chromavision ACIS II system. Ten high power field (x400) from 10 randomly selected neoplastic follicles were counted. The average percentage of Ki-67+ and Skp2+ cells from each case was used for statistical analysis (ANOVA). Counting was carried out by a pathologist blinded to the previously assigned morphologic grade.

Results: The table summarizes the results (±SEM). The Ki-67+% and Skp2+% cells significantly differ among the different grades of FL. The higher grade of FL is associated with the higher Ki-67+% and Skp2+% cells. The overall Skp2+% cells are significantly lower than Ki-67+% cells in the same grade of FL. Statistical significance is observed in Ki67+% cells only between G1 FL versus G3 FL. In contrast, statistical significance is noted in Skp2+% cells between G1 FL and G3 FL and between G2 FL and G3 FL.

Table 1

Grade/Cases	Ki-67 (+%)	Skp-2 (+%)
G1/21	8.34±1.91	4.87±0.90
G2/7	21.90±8.25	5.91±1.87*
G3/17	33.85±5.01*	16.27±3.58*

*p<0.05

Conclusions: Our preliminary data suggest that the Skp2 expression has a better correlation with the grades of FL, compared with the Ki-67. Compared to the traditional methods, quantitation of the Skp2 expression using a quantitative image analysis system appears to be a useful and objective approach in grading FL.

1203 Angioimmunoblastic T-Cell Lymphoma: Histological Progression Is Associated with EBV and HHV6 Infection

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Background: Angioimmunoblastic T-cell lymphoma (AITL) is histologically characterized by the effacement of the normal lymph node structure by a polymorphic infiltrate of atypical CD10 positive T-cells, various inflammatory cells, a prominent vascular network and follicular dendritic meshwork. According to the histological presentation, AITL is divided into three overlapping patterns with pattern I showing minimal effacement of the lymph node structure at one end of the spectrum, and pattern III displaying a total obliteration of the lymph node structure at the other. The histological features and presence of EBV and HHV6B in AITL suggest that these infectious agents play a role in the pathogenesis of this disease despite their presence in the reactive B-cells rather than in the malignant T-cells. To investigate this, we correlated the histological pattern of AITL with infection by both viruses.

Design: The histology and immunophenotype of 33 cases of AITL were reviewed. HHV6B and EBV were detected by viral specific PCR of DNA samples prepared from lymphoma tissue specimens. EBV load was further quantified by real-time PCR. The histological pattern of AITL was correlated with EBV and HHV6B infection.

Results: Among the 33 cases of AITL examined, 24 showed histological pattern III, while 7 and 2 displayed pattern II and I respectively. HHV6B was found in 13/33 (39%), exclusively pattern III cases. EBV was detected in 27/33 (82%) cases, including 11 cases positive for HHV6B. Although there was no apparent difference in EBV positivity among groups showing different histological patterns, real-time PCR revealed a significant association between EBV load and histological pattern. A high EBV load (>50 copies/1000 cells) was seen in 14/24 cases with histological pattern III, but only in 1/7 cases with pattern II and in 0/2 cases with pattern I. This association appeared to be independent of HHV6B infection as the same trend was seen in HHV6B negative cases.

Conclusions: HHV6B infection and high load of EBV infection are significantly associated with AITL that showed histological pattern III. The results suggest that the viral infection may play an important role in the histological progression of this malignant disease.

Infections

1204 Duodenal and Gastric Mucosal Histopathologic Changes in Patients with Giardiasis

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Background: Giardiasis is a cosmopolitan disease, its prevalence varies from 2-7% in developed countries to 40% in developing countries. In Egypt; the rate is reported around 15% in patients with Gastrointestinal complaints. Reported histopathological changes in duodenum and gastric mucosa varies from 3.4% to 50% in different studies.

Design: 32 stool positive cases of Giardiasis were studied; biopsies were taken from the duodenum and stomach (body and antrum) from each patient. In addition to studying the pathological changes; duodenal biopsies were studied for the presence of Giardia Lamblia and gastric biopsies for Helicobacter Pylori

Results: Giardia Lamblia were detected in 13 of 32 duodenal biopsies (40.6%0, chronic inflammation in 27 cases (84.4%), Partial villous atrophy in 13 (40.6%) and fibrosis in 6 cases (15.6%). Pathologic changes were more evident in Giardia positive compared with Giardia negative biopsies (for chronic inflammation 92.3% versus 78.9%, p= 0.625, for partial villous atrophy 46.2% versus 36.9% p= 0.720 and for fibrosis 23.7% versus 10.5% p= 0.374, for Giardia positive and negative biopsies respectively). Histopathologic changes found were active chronic gastritis in 25 of 26 (96.2%) antral biopsies, Helicobacter pylori in 18, (69.2%), follicular gastritis in 11 (42.3%), mild fibrosis in 7 (26.9%) and mild atrophy in 5 (19.2%). Similar but less changes were seen in biopsies from the body of stomach, and changes in the antrum were unrelated to the presence of Giardia in the duodenum.

Conclusions: Duodenal histopathological changes (duodenal biopsy positive more than negative) should be considered in explaining clinical gastrointestinal manifestations in such patients. The observed association between Helicobacter pylori, antral active chronic gastritis in patients with Giardiasis requires further confirmation.

1205 Mean Neutrophil Volume: A New and Reliable Indicator for Acute Infection

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Background: The correct diagnosis of septicemia is important for proper patient management. Laboratory evaluation of sepsis includes blood culture and complete blood count with differential (CBCD). However, blood culture results are only available within several days. White blood cell counts (WBC) are readily available in the automated CBCD, but identification of neutrophil morphologic changes, such as toxic granulation and vacuolization, usually requires manual examination, which is labor intensive and subjective. We have demonstrated that such morphologic changes can be quantitatively measured with the automated hematology analyzer (Coulter LH 750) with VCS technology. In this study, we investigate the clinical utility of neutrophil volume changes in septic patients using the Coulter LH 750.

Design: We retrospectively analyzed data from 69 patients (M/F=36/33; Mean age = 52 yrs; WBC: 1,700/mL – 39,200/mL; Mean = 12,750/mL) with positive blood cultures. Patients whose blood cultures yielded bacteria likely to be contaminants, like coagulase negative Staphylococci, were excluded from the study. The patients were further subdivided into three groups based on WBC: group I (N=31; WBC: 1,700/mL - 11,000/mL; Mean = 6,600/mL), group II (N=18; WBC: 11,000/mL - 15,000/mL; Mean = 12,700/mL), and group III (N=20; WBC: 15,000/mL – 39,200/mL; Mean = 22,300/mL). Thirty-three controls (M/F=10/23; Mean age = 51 yrs; WBC: 4,100/mL –10,900/mL; Mean = 6,930/mL) were selected from patients with CBCD within normal limits and no signs of infection. The mean neutrophil volume (MNV) was measured by Coulter LH 750, and reflects the mean channel numbers of direct current impedance generated by each individual cell passing through the aperture. Statistical analysis was performed using the Student t test.

Results: A significant increase in the MNV was observed in the septic patients compared to controls (156 ± 13 vs 143 ± 4; $p < 0.001$). Such increase in the MNV was observed even in group I with WBC in the low or normal range (152 ± 13, $p < 0.01$). Progressive increases were seen in groups II (MNV = 157 ± 15; $p < 0.01$) and III (MNV = 161 ± 9; $p < 0.001$).

Conclusions: The MNV of reactive neutrophils is significantly elevated in septic patients, even in cases with low or normal WBC, indicating that the MNV may be a more sensitive indicator for acute infection. Since it is a quantitative parameter automatically generated during CBCD, we believe that the MNV has potential to be used as one of the indicators in the diagnosis of acute infection.

1206 Identification of Biomarkers for Systemic Streptococcus and Staphylococcus Infections by Proteomic Analysis

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Background: Treatment of bacterial infection is a time sensitive matter. Early identification of pathogenic bacteria is still a great challenge by using traditional methods. A spectrum of changes in protein patterns in serum has been associated with infection. With the development of 'surface–enhanced laser desorption/ionization time-of-flight mass spectrometry' (SELDI-TOF MS), rapid dectection and characterization of the protein patterns can be achieved using small amounts of sample. Analysis of the protein patterns in correlation with current standard diagnostic information allows the identification of biomarkers for infectious diseases, and to improve patient care.

Design: The serum samples from patients with culture-positive bacterial infection were used for proteomic analysis by SELDI-TOF MS on CM10 chips. We analyzed 13 patients with Staphylococcus aureus infection, 2 with Coagulase-negative Staphylococcus, 3 with Group B Streptococcus, and 1 with Group D Streptococcus. The data were analyzed using ProteinChip software and Biomarker Wizard (Ciphergen Biosystems, Fremont, CA).

Results: 160 clusters of peaks (proteins or peptides) ranging 1 kDa – 60 kDa were identified. 64 of them were identified as biomarkers i.e., peaks whose expressions varied significantly among the types of bacteremia. Though the sample size is limited, preliminary classification trees could be built to distinguish the four types of bacteremia, with > 90% success rate of prediction. Peaks at approximate masses 1006, 1040, 12621, and 14719 Da are important for this classification.

Conclusions: We have analyzed serum protein profiles from 19 patients with bacteremia, by proteomic analysis (SELDI-TOF MS). Potential biomarkers are identified from different bacterial infection samples. Further characterization of these protein biomarkers could eventually contribute to the rapid and early identification of pathogens, and enable the early anti-bacterial treatments.

1207 Spectrum of Pneumocystis Infection in the Setting of Transplantation

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Background: *Pneumocystis* pneumonia is a significant cause of morbidity and mortality in immunocompromised individuals, but is a relatively infrequent complication of chronic immunosuppression in the setting of organ transplantation.

Design: Cases of pulmonary *Pneumocystis jiroveci* (previously *Pneumocystis carinii*) infection at the University Health Network (Toronto) from July 2001 to September 2004 were reviewed.

Results: Twenty-nine cases of pulmonary *P. jiroveci* infection were identified. Of these, 21 cases were associated with HIV/AIDS, and two with malignancy. Another two were found in pre-transplant patients with pulmonary fibrosis on chronic steroids, while four cases were identified in post-transplant recipients (13.8%). Two of these were present in patients following bone marrow transplantation, and two were beneficiaries of a heart transplant. The histopathologic spectrum of pulmonary *Pneumocystis* infection in the transplant-related cases ranged from edema with classic frothy exudates, to diffuse alveolar damage, to necrotizing and calcifying granulomatous forms. In a number of cases, especially those associated with granulomatous lesions, *Pneumocystis* was not suspected clinically, and conventional means of identifying *Pneumocystis* infection (e.g. bronchoscopy, bronchoalveolar lavage) were non-diagnostic, ultimately necessitating open lung biopsy.

Conclusions: *Pneumocystis* infection in immunosuppressed individuals may occur following both hematologic and solid organ transplantation, and is characterized by a broad histopathologic spectrum of injury that may be diagnostically challenging. To avoid missing this infrequent but treatable complication, staining with methenamine silver should be performed even in cases where the pattern of injury may not immediately suggest *Pneumocystis* infection.

1208 Tissue Injury in the Murine Model of Granulocytic Anaplasmosis Relates to Host Innate Immune Response and Not Pathogen Load

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Background: Human granulocytic anaplasmosis is caused by the neutrophilic rickettsia *Anaplasma phagocytophilum*. Humans and animals develop fever, systemic manifestations, pancytopenia, and liver injury, with a case fatality rate of 1%. Predicting severity is difficult and a correlation between infected cell burden and severity/histopathologic injury is not observed. We showed increased histopathologic injury in the mouse model related to interferon-$_{\gamma}$, and also that *A. phagocytophilum* loads are unaltered by innate immunity, including TNF_{α}, NOS2, $gp91^{phox}$, TLR4, TLR2, and MyD88. Thus, we hypothesized *A. phagocytophilum*'s avoidance of innate immune killing is unlinked from responses that cause tissue injury and disease.

Design: We analyzed the histopathology in infected knockout mice (TNF_{α}, NOS2, $gp91^{phox}$). All mice were infected by i.p. inoculation with blood from infected (*A. phagocytophilum* MRK strain; 10^4 to 10^6 genome equivalents) or uninfected SCID mice. Tissues were examined by routine $H_{\&}E$ at intervals after infection and were ranked for degree of severity. Ranks were adjusted for the differing histopathology in uninfected knockout controls. Tissue and blood levels of *A. phagocytophilum* were determined by quantitative PCR previously.

Results: Histopathologic changes peaked between days 7-21 (r=-.52 to -.78) and were inflammatory, with infiltration by histiocytes, lymphocytes, and neutrophils; apoptosis was observed in some hepatocytes, and marked mononuclear cell hypercellularity and foamy histiocyte infiltrates were also observed in spleen and lymph node. Compared to infected wild-type mouse controls, significantly less histopathologic injury was seen in liver at early timepoints (3-21d) in infected TNF_{α} and NOS2 knockout mice ($p < .01$ and $< .04$, respectively), whereas tissue injury was greater in $gp91^{phox}$ knockout mice ($p < .05$).

Conclusions: Despite no significant differences in bacterial loads in control vs. knockout mice, reduced histopathologic injury to liver and lung demonstrate a role for innate immunity in tissue injury and disease with *A. phagocytophilum*. Additional studies of the innate immune response, the role of NO and phagocyte oxidase, and the pathogen-associated molecules responsible for triggering the responses are needed.

1209 Parasitic Infections in a Peruvian Chinese Coolie's Mummy from 1853

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Background: This Chinese coolie was a contract laborer for work in the guano fertilizer mining on islands off the Peruvian coast. He was a chance find in a modern naval base that was originally a quarantine immigration station with a hospital for sick immigrants since 1833.

Design: He was autopsied and his organs were studied using modern histopathological techniques.

Results: The immediate cause of death was an acute diarrhea due to Balantidium coli. Hundreds of these large ciliated protozoa were found in the colon. He also suffered from a pneumonia and the lung fluke Paragonimus was present in his lungs. His liver had multiple nests of Schistosoma japonicum eggs, but he probably had a relatively good liver function.

Conclusions: Although this man died over 150 years ago such studies are a pertinent reminder of diseases today entering the United States due to extensive population movements from one geographic area to another. Numerous infections never seen here before may come in with immigrants and be dormant for years before reactivating. Eleven cases of lung fluke were identified in 1980 in Minnesota among Hmong refugees, but they were initially diagnosed and treated as tuberculosis.

1210 Pulmonary Histopathologic and Immunohistochemical Features of Influenza Cases during the 2003-2004 Season

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Background: In response to early reports of pediatric deaths during the 2003-04 influenza season, CDC initiated enhanced national surveillance for influenza-associated deaths among children and received specimens from106 patients with suspect influenza.

Design: We describe the pathologic and immunohistochemical (IHC) features of lung tissue specimens from 55 patients with laboratory-confirmed influenza. For the IHC assays, we used monoclonal anti-nucleoprotein antibodies for influenza A and B viruses.

Results: Samples belonged to 7 (13%) children < 6 months old, 9 (16%) between 6 and 23 months, 15 (27%) between 2 and 5 years, 10 (18%) between 6 and 12, and 8 (15%) between 13 and 17 years old. Six patients (11%) were adults. There were 26 males (47%). Positive testing for influenza in these 55 cases included antigen immunoassays with enzyme or fluorescent labeling in 46, IHC in 30, cultures in 14, and reverse

transcription-polymerase chain reaction in 2. Peribronchial and peritracheal mononuclear inflammatory infiltrate was present in 50 cases (91%), interstitial inflammation in 26 (47%), bronchopneumonia in 23 (42%), and intraalveolar hemorrhage in 11 (20%). IHC showed viral influenza A antigens in 29 cases and influenza B antigens in 1 case. IHC provided the only confirmatory diagnostic test in 8 cases (15%). By using IHC, viral antigens were observed focally in bronchial epithelial cells and cells lining ducts to mucous glands in trachea and centrally located bronchi. Antigens were rarely observed in desquamated ciliated cells in the alveolar lumen or interstitial mononuclear cells. Negative IHC results occurred when there was extensive necrosis or desquamation of bronchial epithelial cells or if only peripheral lung samples were studied.

Conclusions: Tracheitis and bronchitis were the most frequent pathologic features of influenza infections during the 2003-04 season. IHC positive results were obtained from 55% of cases, being the only influenza diagnostic test in 15% of cases. Because IHC staining is very focal, we recommend studying at least 6 sections of perihilar lung tissue containing primary or segmental bronchi.

1211 Diagnosis of Group A Streptococcal Infections by Using Immunohistochemical and Molecular Assays

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Background: Increasing numbers of cases of acute rheumatic fever, necrotizing soft tissue infections, and toxic shock syndrome suggest there is an increasing severity of group A streptococcal (GAS) infections. GAS may not isolated if antibiotic treatment has been given. Formalin fixed samples (FFS) may be the only specimen available if cultures are not obtained or negative.

Design: We applied immunohistochemical (IHC) assays to FFS from 23 patients with invasive GAS disease. Polyclonal antibody to GAS carbohydrate used in the assay reacted against *S. pyogenes* and did not cross react against group B *Streptococcus*, *S. aureus*, *H. influenzae, N. meningitidis*, *H.pylori*, *C. burnetti*, and *L.monocytogenes* or against tissue sections from patients with confirmed *S. pneumoniae* or *L.pneumophila* pneumonia. A hemi-nested PCR assay targeting the sepB gene which encodes for the pyrogenic endotoxin B of *S. pyogenes* was applied to FFS from patients who had not been cultured or had negative culture results.

Results: *S. pyogenes* cultures were available in 13 cases and 8 others showed PCR evidence of *S. pyogenes*, including 3 cases in which antibiotics were given and cultures were negative. IHC showed GAS antigens in the 23 patients. The GAS antigens and streptococci were mostly seen within monocytes but some were also seen extracellularly. Distribution of GAS antigens and streptococci included: 6 patients with septicemia and a primary source of infection (3 pneumonias, 2 cutaneous, 1 uterine), 5 patients with septicemia but unknown source, 3 patients with pneumonias, 4 patients with upper respiratory infections (2 tonsilitis, 2 laryngeal abscesses), and 5 patients with cutaneous soft tissue infections. In 2 patients with necrotizing fasciitis, the diagnosis was only accomplished by using IHC, while in 1 patient with pneumonia, the diagnosis was achieved by using the IHC assay on a pleural fluid cell block.

Conclusions: IHC allowed specific diagnosis of GAS infections and determined the distribution of streptococci and GAS antigens in tissues. PCR assays of paraffin-embedded material can also be used to confirm the diagnosis. These methods are valuable in diagnosing cases in which cultures show no growth because of antibiotic treatment or for which only formalin-fixed tissues are available.

1212 Development of a Diagnostic PCR Assay for Molecular Biogrouping of Pathogenic *Yersinia enterocolitica*

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Background: *Yersinia enterocolitica* (YE) is a food-borne bacteria implicated in the pathogenesis of ileocolitis, granulomatous appendicitis, and malakoplakia. Some epidemic YE infections have been linked to either biogroup 1B (high virulence serovars) or 2/5 (low virulence serovars). Our goal was to develop a molecular biogrouping assay for use with both archival specimens and cultured bacteria.

Design: Seven different pathogenic ATCC/CDC known YE serovars (four biogroup 1B and three biogroup 2/5), and six archival YE positive human cases were studied. Polymerase chain reaction was performed on the extracted DNA using novel primer pairs targeting a 182-bp region of the 23s rRNA gene for biogroup 1B, and a 204-bp region of the same gene for biogroup 2/5. After gel electrophoresis, the band pattern was evaluated. As the 182-bp fragment is present in both biogroups, yet the 204-bp fragment is present only in low virulence serovars, subjecting samples to both reactions allows biogroup distinction. Sequence analysis was performed to confirm that PCR products matched expected sequences for these gene segments.

Results: The seven ATCC/CDC known culture isolates were appropriately confirmed as four biogroup 1B serovars and three biogroup 2/5 serovars. Of the six archival tissue cases, four yielded DNA corresponding to biogroup 2/5, and two yielded DNA corresponding to biogroup 1B. Histologic correlation of the four low virulence YE cases revealed three granulomatous appendicitis cases, and one malakoplakia. Of the two patients with high virulence biotypes, one had YE sepsis and one had a YE abscess.

Conclusions: Using two novel primer pairs, all seven culture isolates and all six parafin blocks were successfully amplified and biogrouped. This is the first molecular assay developed for pathogenic YE biogrouping, and it is suitable for use with both cultures and archival patient materials. In addition, these data suggest that low virulence YE serovars may be associated with chronic granulomatous infections such as granulomatous appendicitis and malakoplakia, whereas high virulence strains are associated with suppurative inflammation and sepsis. This assay could yield important information linking epidemiology and morphologic patterns of YE infection. Additionally, it could be a useful tool in the review of both ongoing and retrospective food-borne outbreaks.

1213 Human Papillomavirus and Renal Tumors: A Controversial Issue Revisited with Immunohistochemistry and Signal-Amplified In Situ Hybridization for Detection of HPV DNA and P16^{INK4A} in Paraffin Embedded Tissue

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Background: The association between human papillomavirus (HPV) infection and carcinogenesis has long been established in literature, with the strongest evidence for its role in cervical carcinoma. The role of HPV in urological tumors has been investigated and sporadic reports have linked HPV infection to bladder, prostate, renal, penile and testicular cancer. Although less rigorously studied, there are conflicting results about the role of HPV in the development of renal cell carcinoma (RCC). This is the first study to examine 62 renal tumors, including oncocytomas, different histological types of renal carcinomas, collecting duct carcinoma (CDC) and urothelial carcinomas of renal pelvis for detection of HPV DNA and P16, which is a cyclin dependent kinase inhibitor shown to be expressed in cervical dysplasia associated with high risk HPV.

Design: Formalin-fixed, paraffin-embedded tissues from 62 consecutive renal tumors (40 clear cell, 9 papillary, and 3 chromophobe RCCs, 7 oncocytomas, 1 CDC and 2 urothelial carcinomas of renal pelvis) were immunostained with monoclonal antibodies against low and high risk HPV DNA (HPV 6, 11, 16, 18, 31, 33, 42, 51, 52, 56, 58, Dako, clone # K1H8, Carpinteria, CA). Sections were also stained with an immunohistochemical (IHC) antibody to p16^{INK4A} (Dako, code-K5334). Tissue microarray sections of 62 tumors were stained with Signal-Amplified Colorimetric In-Situ Hybridization (SAC- ISH), GenPoint™ method, using biotinylated probes for subtypes 6, 11, 16, 18 (Dako, code-K0620, clone #'s Y1405, Y1406, Y1407, Y1408, Carpinteria CA), which easily allows detection of 1-2 copies of HPV DNA. A patchy signal unevenly distributed over tumor cell nuclei is considered positive for low and high risk HPV and nuclear or cytoplasmic staining is considered positive for P16 by IHC and nuclear dot-like signal is considered positive by SAC-ISH.

Results: No nuclear staining was found for HPV-DNA with IHC and SAC-ISH and no nuclear or cytoplasmic staining was found for P16 with IHC in any type of renal tumors.

Conclusions: Our results suggest that HPV does not seem to play a role in the development of different types of renal cell carcinomas, collecting duct carcinomas, urothelial carcinomas of renal pelvis or oncocytomas.

1214 Complete Whole Body Mapping of Tissue Reservoirs of Human Herpesvirus Infection with a Pan-Herpes PCR Technique

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Background: There are a total of eight different and widely distributed human herpesviruses (HSV1, HSV2, VZV, EBV, CMV, HHV6, HHV7, HHV8). Despite the fact that all herpesviruses persist lifelong in the human host, no comprehensive survey of all sites of herpesvirus persistence has previously been reported.

Design: We recently developed a novel PCR method for detection and species identification of all eight human herpesviruses with a set of consensus primers and species-specific probes to the highly conserved herpesvirus DNA polymerase gene (Hudnall et al. J Virol Meth 2004). DNA from approximately 40 fresh specimens representing all major organs and tissues from seven unrestricted adult autopsies was collected. Causes of death were all unrelated to herpesvirus infection. DNA was subjected to pan-herpes PCR with digoxigenin-labeled dUTP. Labeled products were hybridized to nylon membranes previously labeled with species-specific probes. Hybridization signals were detected by immunoalkaline phosphatase-induced chemiluminescence.

Results: HSV-1 was often detected in non-CNS neural tissues (peripheral nerve, sensory ganglia, spinal cord), nasal mucosa, cartilage, marrow, skin, and bone. HSV-2 was detected only once in anal tissue. VZV was rarely detected in non-CNS neural tissues, nasal mucosa, cartilage, bone, and adrenal gland. EBV was commonly detected in numerous tissues, including tongue, tonsil, nasal mucosa, cervical node, small and large intestine, rectum, liver. spleen, and thymus. CMV was occasionally found in peripheral nerve, nasal mucosa, trachea, lung, thyroid, large intestine, liver, bladder, urethra, and anus. HHV6 was commonly found in parotid, submandibular gland, stomach, small and large intestine, liver, spleen, pancreas, kidney, and abdominal node. HHV7 was not found in any tissue, while HHV8 was found only in two nodes (mediastinal and cervical).

Conclusions: EBV was commonly found in oral, nasal, lymphoid, and gastrointestinal tissues. HSV-1 was found in a variety of tissues, including oral, nasal, and neural tissues, marrow, cartilage, and bone. HSV-2 on the other hand was found only in the anus. VZV, although less common, had a distribution like that of HSV-1. CMV was found primarily in tracheobronchial and gastrointestinal tissues, while HHV-6 was surprisingly common in gastrointestinal sites. HHV7 was undetected and HHV8 was detected in two lymph nodes only. To our knowledge, these results represent the most comprehensive mapping of tissue distribution of all eight human herpesviruses yet produced.

1215 Capsule Intact(Cap I) and Deficient(D) Pulmonary Cryptococcal(Crypt) Infections: Histologic and Radiologic Correlation

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Background: The pathologic forms of cryptococcosis in the lung have been well recognized since at least the mid 1950s. Crypt organisms are small yeast-like, globose fungi surrounded by the cap, which are acidic polysaccharides and a prime determinant of virulence. The special stains such as mucicamine(Mu), alcian blue(AB), Gomori methenamine silver(GMS) and Fontana-Masson(FM) stains have recently identified Cap D Crypt infection. To better understand the histologic appearance as well as radiologic features of pulmonary cryptococcosis related to Cap, we analyzed Cap I and D pulmonary Crypt infection cases.

Design: 12 cases of pulmonary cryptococcosis were retrieved from the surgical pathology files of the AMC and Seoul National University Hospital from Jan. 1997 to June 2004. The Mu, AB, GMS and FM stains were performed to classify the fungi in all cases. Pathologic and radiologic features were correlated with presence or absence of Cap in Crypt infections. Also we evaluated pathologic features such as granuloma(G) formation, collection of giant cells, severity of inflammation, distribution of organisms and presence of necrosis. Cap D organisms were defined when the organisms were positive for GMS and FM, but not or weakly stained by Mu and AB stains.

Results: There were 5 men and 7 wemen and all the lesions were detected by CT scans as mass-like consolidation, multifocal nodules or ground-glass opacity. Based on Mu and AB stain results, Cryptococcus spp. were divided into the Cap I(6 cases) and Cap D(6) forms. Of 6 cases of Cap I, 1 case showed many Gs(16.7%) but the others had a few Gs(83.3%), and 3 cases revealed necrosis(50%). Of 6 cases of Cap D, 2 cases revealed many Gs(33.3%) and the others had a few Gs(66.7%), but none of them showed necrosis.. Fungal organisms were observed both in the giant cells and free-floating in both groups. There was no significant difference in severity of inflammation and collections of giant cells between two groups. In addition, there was no significant difference in radiologic features between two groups except for more cavity formation in Cap I group by CT scan.

Conclusions: Although there was more G formation in Cap D and more necrosis in Cap I Crypt infection, these features were overlapped between two groups. In addition, there were no specific radiologic features to distinguish Cap D from I ones. Therefore, special stains including GMS, FM, Mu and AB are mandatory to render a diagnosis of Cap I vs.D Crypt infections in the lung.

1216 Surgical Pathology of Arthropods: Case Series Review and Development of a Diagnostic Algorithm

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Background: The pathologic diagnosis of arthropods can be difficult. On gross examination, arthropod larvae can be mistaken for helminths, and non-pathogenic free-living arthropods sometimes are mistaken for parasites. When examining tissue sections, diagnostic structures are often distorted or absent, host tissue response can be non-specific, and contaminants can mimic exoskeletal structures, complicating efforts at an accurate diagnosis.

Design: We reviewed 106 cases of arthropods submitted for pathology examination over a 5-year period, in an effort to define diagnostic features and to develop a common, practical diagnostic algorithm that could be used by general practice surgical pathologists.

Results: The cases included 24 fly larvae (myiasis), 43 ticks, 8 fleas (tungiasis), 14 mites, 5 unclassified arthropods, 3 contaminants, and 9 free-living (non-pathogenic) arthropods. The misdiagnosis of myiasis or tungiasis as parasitic helminthic infection was the most common mistake made by referring pathologists. In approaching cases potentially involving arthropods, a simple and rapid three-stage approach is proposed. Assessment for presence or absence of an arthropod is based on observation of exoskeleton, skeletal muscle or tracheal structures. Key morphologic features can then be used to classify the organism phylogenetically. Finally, a multifaceted approach, including clinical history, must be used to determine the pathogenicity of a given organism.

Conclusions: The routine use of this algorithm should enable rapid, accurate, and unambiguous results, while reducing the incidence of misdiagnosis based on the presence of contaminants or the misclassification of organisms.

1217 Clinical Evaluation of the Use of Perservcyt®ThinPrep Liquid Medium (Cytyc Corp., Boxborough, MA) Versus Conventional Slide Preparation for the Morphologic Identification of Fungal Pulmonary Pathogens from Bronchoalveolar Lavage (BAL) Specimens

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Background: Laboratory culture for *Histoplasma capsulatum* and many fungal pulmonary pathogens is cumbersome and not available at all for others such as *Pneumocystis carinii*. Conventional slide preparation with or without special fungal stains has traditionally been an acceptable method for the morphologic identification of pathogenic fungi from BAL specimens. Because conventional slide preparation is tedious, costly, not available at all times and may lack sensitivity, we evaluated the use of ThinPrep(TP)-prepared slides for screening these specimens.

Design: Fifty-six samples from immunocompromised patients have been tested to date. Conventional screening include centrifugation and pelleting a 20 ml aliquot of the BAL specimen and then staining with DiffQuik (DQ), Giemsa and Gomori Methenamine Silver stain (GMS). For TP processing, 5 ml of BAL specimen is placed into a TP vial and the entire contents are centrifuged. An aliquot is then placed in a second TP vial and processed on the TP 2000 system according to manufacturer's instruction. The TP slides are then stained with Pap, DQ and GMS

Results: Of the 56 specimens processed, 21 fungal pathogens were recovered from 17 patients. Ten specimens (9 patients) were positive for *Pneumocystis carinii*; 3 (3 patients) were positive for *Candida albicans*; 3 (2 patients) were positive for *Aspergillus flavus*; 2 (1 patient) were positive for *Blastomyces dermatitidis*; and 3 (3 patients) were positive for *Histoplasma capsulatum*. These results were identical to traditionally prepared slides except for one case of *Candida albicans* seen only on traditional DQ.

Conclusions: Preliminary results from this study suggest that detection of fungal pathogens using TP processing is equivalent to traditional processing. TP processing allows specimens to be processed and screened by cytotechnologists before being reviewed by a pathologist. TP also allows for delayed processing of up to 6 weeks without specimen degradation. Pap stain of TP processed material is an excellent alternative for staining fungal pathogens with enhanced cellular distribution and clarity. Cytology of the cells and microbial pathogens is improved in TP processed specimens.

1218 Histopathology and Immunohistochemical Localization of Influenzavirus in Patients with Fatal Influenzavirus B

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Background: Influenzaviruses are segmented negative-sense RNA viruses that are typed on the basis of antigenic differences among nucleoprotein and matrix proteins. Influenza A and B are the two types of influenzaviruses that cause epidemic human disease. Accuracy of a clinical diagnosis of influenza may be limited because many other viral and bacterial pathogens can cause influenza-like illnesses. Previous studies have described immunohistochemical (IHC) detection of influenzavirus A in patient tissues; herein we describe a novel IHC assay for the detection of influenzavirus B.

Design: Formalin-fixed, paraffin-embedded tissue specimens from patients with laboratory-confirmed influenza B were evaluated by using routine stains and by an IHC stain to identify specifically viral antigens of influenzavirus B

Results: Tissue specimens from 9 patients with fatal influenza B infection were evaluated at the Centers for Disease Control and Prevention during 1999-2004. Patients included 8 females and 1 male from 6 states and ranged in age from 2-20 years (median, 6 years). Influenza B was confirmed by the following tests, alone or in combination: direct fluorescent antibody (2); culture (2), rapid antigen detection (1) and IHC (8). Four IHC-positive patients were also positive by another assay and 1 culture-positive patient was IHC-negative. For 3 patients IHC was the sole confirmatory assay. Frequent histopathologic features included tracheitis, bronchitis, bronchiolitis, pulmonary congestion and edema, intraalveolar hemorrhage, and interstitial pneumonitis. IHC staining demonstrated antigens of influenzavirus B in ciliated respiratory epithlium in bronchi and trachea, and less often in mucous gland epithelium and detached epithelial cells in bronchioles. Despite extensive histopathology, antigens were generally sparse and focally distributed in patient tissues, similar to descriptions of the distribution of influenzavirus A.

Conclusions: Histopathological features of fatal influenza B are generally nonspecific and often appear out of proportion to the quantity of viral antigen detected by IHC. Viral antigens are most often localized to larger airways and adequate sampling of these tissues is crucial to establish an IHC-based diagnosis. Because of the broad differential diagnosis suggested by the histopathologic findings of influenzaviruses A and B, IHC testing provides an important confirmatory method for diagnosing fatal influenzavirus infections.

1219 Pathology of Fatal Lymphocytic Choriomeningitis Virus Infection in Multiple Organ Transplant Recipients from a Common Donor

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Background: Lymphocytic choriomeningitis virus (LCMV), a rodent-borne arenavirus, causes a spectrum of clinical manifestations in human hosts; however, case fatality rates of this infection are characteristically <1%. Histopathologic studies of disease in humans are limited to descriptions of CNS pathology and no data are available to describe the cellular targets and distribution of LCMV in human tissues. During November 2003-January 2004, 4 recipients who received lungs, liver, or kidneys from a common donor died 9-76 days following transplant. Clinical suspicion of a common infectious etiology prompted a multidisciplinary laboratory investigation by the Centers for Disease Control and Prevention that subsequently identified disseminated LCMV in 3 of 4 recipients.

Design: Formalin-fixed, paraffin-embedded tissues were evaluated by routine histopathological and special stains, and by various immunohistochemical (IHC) stains for viral pathogens. CSF specimens from several patients were inoculated into Vero cells and cultures were evaluated by electron microscopy (EM), indirect fluorescent antibody (IFA) staining, and RT-PCR.

Results: Histopathology was most notable for necrosis without significant inflammation in transplanted organs. Available donor tissues, including CNS, showed no inflammatory infiltrates. Initial IHC stains for various flaviviruses, adenoviruses, and herpesviruses were negative. Cell culture isolates from two patients were subsequently identified as an arenavirus by EM and IFA and further characterized as identical strains of a unique genotype of LCMV by RT-PCR. IHC staining demonstrated LCMV antigens in various tissues, including liver, adrenals, lung, spleen, kidney, or skin of three recipients. No viral antigens were identified in any CNS tissues or in remaining donor tissues.

Conclusions: Ascertainment of the cause of death for these patients was accomplished rapidly because of clinical acuity that recognized the potential for a common source of

infection of the recipients and a multidisciplinary laboratory approach to establish an etiological diagnosis. The pathology and IHC staining of transplant-associated LCMV infection is most reminiscent of Lassa fever. The contributory role of immunosuppression in the pathology and pathogenesis of LCM in these patients deserves further study.

1220 Howell-Jolly Body-Like Inclusions in the Neutrophils of HIV Positive Patients Are Associated with Low CD4 Counts

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Background: Howell-Jolly body-like inclusions are discrete intracytoplasmic inclusions which have been previously observed in the neutrophils of HIV positive individuals. The inclusions are morphologically and structurally similar to red blood cell Howell-Jolly bodies; consisting of remnant nuclear material and appearing as small, densely basophilic inclusions. Little is known of their clinical significance, although it has been suggested that the appearance of Howell-Jolly body-like inclusions is related to antiviral medications. We evaluated the incidence of the inclusions in HIV positive individuals and correlated these results with clinical parameters including CD4 count, which has not previously been reported.

Design: Peripheral blood smears from 18 consecutive HIV positive patients and 20 consecutive HIV negative patients who underwent bone marrow biopsy were reviewed. The entire thin portion of the peripheral blood smear was reviewed for the presence of neutrophil cytoplasmic inclusions. A Feulgen reaction was performed to confirm that the inclusions consisted of nuclear material. Concurrent clinical information was collected including: HIV status, therapeutic regimen, viral load, and CD4 count.

Results: Of the 18 HIV positive patients, neutrophil inclusions were identified in 10 individuals (55%). CD4 counts were significantly lower ($P<0.05$) in inclusion-positive cases (mean 12.86 cells/mm3) versus the inclusion-negative cases (mean 226.7 cells/mm3). HIV RNA viral loads were not significantly different between the inclusion-positive (mean 178,200 copies/ml) and inclusion-negative groups (mean 119,400 copies/ml). Antiretroviral therapy did not show a statistical relationship with the presence of inclusions (30% of inclusion-positive vs. 63% of inclusion-negative receiving antiretroviral therapy). No inclusions were identified in the 20 HIV negative patients.

Conclusions: The findings suggest that Howell-Jolly body-like inclusions are not uncommon in HIV positive individuals. It appears that the inclusions are not related to antiviral medication or viral load. A relationship does appear to exist between lower CD4 counts and the appearance of inclusions. This shows that in HIV positive individuals, Howell-Jolly body-like inclusions correlate with the degree of immune suppression.

1221 Correlation of Histology, Human Papillomavirus Detection, and Viral Load in Laryngeal Papillomas in Childhood

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Background: Laryngeal papillomas are lesions in children associated with human papillomavirus (HPV) infection that have a high recurrence rate. The histologic correlates of these lesions as well as viral load is not well understood.

Design: The purpose of this study was to analyze laryngeal papillomas in children for HPV by in situ hybridization (for productive infection) and PCR in situ and correlate this with the histologic findings.

Results: HPV DNA was detected by in situ hybridization (Ventana Medical Systems) in 29/47 cases (62%); all cases were HPV 6 or 11 positive. We compared the presence of keratohyaline granules, non-uniform perinuclear halos, marked papillomatosis, and marked acanthosis in the HPV positive and negative cases. There was a statistically significant increase in the presence of keratohyaline granules (22/29 -76% vs 9/18 - 50%), non-uniform perinuclear halos (20/29 – 69% vs 3/18 – 17%), and marked papillomatosis (22/29- 76% vs 6/18 – 33%) in the viral positive cases. The viral load was low (defined as less than 10 positive cells per tissue with a corresponding weak signal) in 18/29 (62%) of the viral positive cases; in comparison a high viral load was evident in 17/20 (85%) vulvar condylomas. The viral negative cases were tested for HPV by PCR in situ hybridization using primers for HPV 6 and 11. The detection of HPV increased to 38/47 (81%) after PCR amplification.

Conclusions: It is concluded that larygeal papillomas in childhood are characterized, in general, by a relatively low HPV viral load and that the viral positive cases are associated with keratohyaline granules, non-uniform perinuclear halos, and marked papillomatosis.

1222 Histopathology of the Liver in Untreated Human Immunodeficiency Virus and Hepatitis C (HIV/HCV) Coinfected Patients in a County Hospital

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Background: Previous studies show that patients with HIV and HCV coinfection are at increased risk for progression to cirrhosis compared with patients with HCV infection alone, but the causes are unclear. CD4 cell depletion, cholestatic hepatitis and certain antiretroviral therapies in HIV/HCV patients, and interleukin producing plasma cells in chronic viral hepatitis are suggested as possible causes for fibrosis progression. We studied histopathologic and relevant clinical features of untreated HIV/HCV coinfected patients seen in a county hospital.

Design: Liver biopsy material from 2001 to 2004 was available from 29 HIV/HCV patients. Data obtained from those biopsies were compared to the results of biopsies obtained from 67 HCV patients. Clinical data included CD4 count and HIV RNA load. Sections were stained with H&E, trichrome and methyl-green-pyronin (for easier visualization of plasma cells). All biopsies were assessed for grade, stage (Ludwig and Batts), and other significant pathologic findings. Type and distribution of inflammatory infiltrate with plasma cell count was performed in 26 HIV/HCV patients and 17 HCV patients.

Results: Comparison of HIV/HCV group vs HCV group: 22/29 vs 36/67 males (75.9% vs 53.7%); mean age of 45.9 vs 50.7; 51.7% vs 61.2% African Americans, 34.5% vs 19.4% Hispanics and 13.8% vs 19.4% White. Mean HIVRNA load was 17556.2 copies/ml; mean CD4 count was 372.2 cells/μl (ranged from 50-945). HIV/HCV group had significantly less inflammatory activity (lower grade than HCV group alone, $p=0.023$), while there was no difference in degree of fibrosis. Due to depletion of lymphocytic infiltrate in the coinfected group, plasma cells were more apparent in HIV/HCV group. However, number of plasma cells per portal area was significantly lower in HIV/HCV group than in HCV alone (14.13 ± 10.02 vs 23.7 ± 10.4, $p=0.04$). There was no correlation between CD4 count or HIVRNA load and grade, plasma cell number and degree of fibrosis. None of the HIV/HCV patients had an opportunistic infection. No cholestatic hepatitis is seen in either group.

Conclusions: HIV coinfection with HCV is associated with less inflammatory activity (lower grade) due to depletion of lymphocytic and plasma cell infiltrate. There is no correlation between CD4 count and degree of inflammation or fibrosis. This suggests that the mechanism of hepatic fibrosis in HIV/HCV coinfection is independent of HIV disease status, inflammatory activity / grade, or plasma cell number.

1223 Pathologic Studies of a Variant Creutzfeldt-Jakob Disease Case in the United States

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Background: A 25-year-old Florida woman with variant Creutzfeldt-Jakob disease (vCJD) died in June 2004. The patient had lived in the United Kingdom during the period of highest risk for human exposure to the agent causing bovine spongiform encephalopathy and was believed to have been infected between 1980 and 1992 before moving to the United States. The patient was the first known person with vCJD to reside in the United States.

Design: A full autopsy was performed by a team from Centers for Disease Control and Prevention (CDC). Precaution and decontamination procedures were followed and tissue samples were collected according to guidelines established by the World Health Organization and CDC. Tissue samples from the central nervous system, all major organs, and other sites, including lymph nodes, tonsil, and muscle, were collected for histopathologic evaluation, immunohistochemical assays (IHC), and other studies.

Results: The neuropathologic changes were distinctive and differed markedly from those caused by non-variant CJD endemic in the United States. Gross examination revealed that the brain was markedly atrophic, with a softened cortical ribbon that sloughed off easily; the white matter was considerably firmer. No significant gross finding was noticed in other major organs. Microscopic examination of brain tissue revealed that the loss of neurons was near complete throughout the cerebral and cerebellar cortices, with numerous "florid" plaques, which are typical of vCJD. These plaques consist of a central amyloid core surrounded by a peripheral rim of spongiform change. IHC using two different antibodies demonstrated diffuse confluent sheets of proteinase-resistant prion protein throughout the cerebral and cerebellar cortices. Focal immunostaining was also observed in lymphoid organs, such as tonsil and spleen. Pathologic studies on other tissue samples from this case-patient are ongoing.

Conclusions: The patient described in this report represents the first vCJD case in a U.S. resident. Although vCJD is a rare disease, pathologists should be familiar with its histopathologic features and diagnostic modalities because of their critical role in diagnosis of and surveillance for this disease. Further pathologic studies should provide valuable insight in understanding the pathogenesis of vCJD.

Kidney

1224 Detection of BK Virus in Laser Capture Microdissected Kidney Biopsies Using Real Time PCR

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Background: The BK virus (BKV) is a major source of infection among renal allograft recipients, and may play an adverse role in long term graft survival. Previous reports of BKV detection using molecular techniques have suffered from poor sensitivity and specificity. There is therefore a need to explore new technologies that could be utilized to improve sensitivity and specificity of BKV detection in tissues. We applied real-time PCR technology to the detection of BKV in H&E stained kidney biopsies, utilizing laser capture microdissection. We believe this represents the first description of the use of real-time PCR for this purpose.

Design: Renal allograft biopsy specimens from a patient with the histologic diagnosis of BKV infection were retrieved. Diagnostic inclusion-bearing cells were microdissected by laser capture microscopy as were normal-appearing glomerular cells. DNA was extracted and real time amplification performed using primers that targeted the large "T" and small "t" regions of the BKV genome. Tubular epithelial and glomerular cells from a control case with no evidence of BKV infection were used as negative controls in a similar reaction.

Results: The presence of BKV was demonstrated in the epithelial cells containing typical viral inclusions, as well as from glomerular cells that appeared normal by histology. The materials from the negative control were completely negative.