Conclusions: In the 8-parameter FCM panel, score ≥ 3 more suggested hMDS. Hypogranularity, increased CD34 might be useful in discriminating hMDS from hypoplastic marrow. Decreased CD71 was frequently found in hMDS with erythoid dysplasia.

Comparison of 8 Flow Parameters in Patients with/without hMDS

Parameters	Normal (A)	Hypocellular (B)	hMDS (C)	*P Value (B vs C)
Hypogranularity	4/41 (9.8%)	1/14 (7.1%)	7/10 (70%)	0.001
CD56	0/41 (0%)	1/14 (7.1%)	3/10 (30%)	0.14
CD10	5/41 (12.1%)	4/14 (28.5%)	6/10 (60%)	0.12
CD64	5/41 (12.1%)	4/14 (28.5%)	3/10 (30%)	0.94
CD13	0/41 (0%)	0/14 (0%)	0/10 (0%)	1
CD33	0/41 (0%)	1/14 (7.1%)	1/10 (10%)	0.80
CD34	1/41 (2.4%)	1/14 (7.1%)	4/10 (40%)	0.05
CD71	2/41 (4.9%)	2/14 (14.3%)	6/10 (60%)	0.02

*Chi-square Test

Table 2. Flow Scoring in Patients with/without hMDS

Scores	Normal (A)	Hypocellular (B)	hMDS (C)
Score <3	41/41 (100%)	12/14 (85.7%)	1/10 (10%)
Score >=3	0/41 (0%)	2/14 (14.3%)	9/10 (90%)
X+/-SD	0.66 +/- 0.69	1.29 +/- 1.14	3.30 +/- 1.06

Student t-test: P Value: A vs B=0.07, B vs C<0.001, A vs C <0.0001

1223 Correlation of ATM Protein Expression with ATM Mutation Status and Overall Survival in Mantle Cell Lymphoma

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Background: Mantle cell lymphoma (MCL) is an aggressive subtype of B-cell non-Hodgkin's lymphoma that has a median survival of 3 to 4 years. One of the frequent abnormalities found in MCL is ATM gene inactivation via genomic deletions in 35-40% of cases; and deleterious point mutations, including missense mutations and protein truncating nonsense mutations that occur in 33-40% of cases. The aim of this study is to determine how protein expression correlates with ATM mutation status and whether protein expression predicts for overall survival in MCL.

Design: A total of 39 cyclin D1 positive MCL cases with a median age of 60.6 years were selected for the study. Immunohistochemical (IHC) staining for ATM was performed on the cases using a tissue microarray (TMA) with three cores for each case. Five micron sections were cut from each TMA and stained with CyclinD1 to evaluate involvement of MCL in the cores. After citrate antigen retrieval, the cores were stained with an antibody to ATM (ATX08, NeoMarkers, CA), which stains normal mantle cells. The percentage of tumor cells stained with each antibody was recorded in 5% increments and the core with the highest percentage of tumor cells stained was used for analysis. Cases were considered positive if more than 20% of the tumor cells showed positive staining for ATM. 30 of the 39 cases had previously collected ATM mutation data. Kaplan Meier analysis was used to estimate median overall survival.

Results: Positive expression of ATM protein was detected in 28 of 39 (71.7%) cases of MCL and in 22 of 30 (73%) cases of MCL with known ATM mutation status. The proportion of positive cells ranged from 40-100% with a mean of 88%. In the latter subset of 22 ATM protein positive MCL cases, 15 (68%) had wild type ATM and 7 (31%) cases had a missense mutation. Of the 8 ATM protein negative MCL cases, 2 (25%) cases had a missense mutation, 5 (62.5%) cases had a nonsense mutation and only one case had wild type ATM. Nonsense mutations were highly predictive of negative ATM protein expression (p=0.001; chi square test). The median overall survival in ATM positive cases and negative cases was 3.25 years and 4.42 years, respectively (p=0.37).

Conclusions: Most of the ATM negative cases are secondary to nonsense mutations which truncate the protein. However, ATM protein expression is not predictive of overall survival in mantle cell lymphoma.

1224 Mast Cell Nuclear Phospho-STAT5 Expression in Systemic Mastocytosis: A Diagnostically Useful Phenotypic Abnormality That Reflects Underlying Biologic Alterations

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Background: Systemic mastocytosis (SM) is characterized by the abnormal proliferation and accumulation of mast cells (MCs). Constitutive activation of KIT, a receptor tyrosine kinase (TK), has been associated with all classifications of SM and >80% of cases harbor a D816V *KIT* mutation. However, other *KIT* mutations may occur, and other TKs (*FIP1L1-PDGFRa)* can be abnormally activated in SM. Signal transducers and activators of transcription (STATs), such as STAT5, are activated (phosphorylated) by these TKs. Phospho-STAT5 (pSTAT5) enters the nucleus and regulates gene transcription. We hypothesized that detection of nuclear pSTAT5 in MCs might reflect TK activation and would be a marker of abnormal MCs in SM.

Design: Archival cases of SM and cutaneous mastocytosis (CM) were obtained. Control tissues for non-neoplastic mast cells included solid tumors (4), bone marrow (BM) containing lymphoplasmacytic lymphoma (1), and normal BM from lymphoma staging procedures (3). Clinical information was reviewed when available and SM patients were categorized by WHO criteria. Expression of tryptase, CD25, and pSTAT5 was evaluated by immunohistochemistry (IHC) on paraffin-embedded tissue. Dual color IHC was performed for tryptase and pSTAT5 to confirm pSTAT5 status when MCs were difficult to identify. *KIT* exon 17 sequencing was perfomed on peripheral blood, BM, and lesional tissue samples when available.

Results: 31 patients were included in the study (23 SM, 8 CM). Cases of SM included: 5 indolent SM, 6 aggressive SM, 6 SM with associated hematologic disorder, and 1 SM with eosinophilic leukemia *(FIP1L1-PDGFRa+*). The remaining 5 cases of SM were not subclassified due to lack of clinical information. Sequence analysis on 11 evaluable SM patients showed D816V (4 patients), D816Y (1 patient), and wild type codon 816 (6 patients). 21/23 SM had CD25+ MCs. 1/8 CM expressed nuclear pSTAT5 in MCs. Interestingly, 21/23 of SM expressed pSTAT5 in MC nuclei while all control tissue MCs were negative for pSTAT5 (P<.0001, Fisher Exact).

Conclusions: Nuclear pSTAT5 in MCs from SM likely reflects abnormal TK activation. Evaluation of pSTAT5 by IHC may serve as a surrogate marker of KIT activation and as a marker of SM (regardless of *KIT* mutation status). We propose nuclear pSTAT5 positivity in MCs as an additional minor phenotypic criterion for diagnosis of SM.

Infections

1225 Strongyloides Colitis Mimics Ulcerative Colitis: A Morphological Differential Diagnosis

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Background: Strongyloides colitis (SC) is a severe form of Strongyloidiasis that carries a mortality rate of 50%. The low index of suspicion due to its relative rarity and morphologic resemblance to Ulcerative colitis (UC) has been a source for histological misdiagnosis at a rate of 44-47%, based on a review of cases from the literature and our institution. We attempt to characterize histological features of SC, contrast it to those of UC, and propose helpful clues to the correct diagnosis of this curable, frequently misdiagnosed and potentially fatal infectious disease.

Design: Twenty four cases including those from the literature and our institution were reviewed. We characterize the gender, mean age, location, and unique clinical and histological findings of this infectious entity. We contrast these findings to that of UC and propose helpful diagnostic criteria for SC.

Results: Patients with SC ranged from 21 to 80 years of age with a median age of 64 years. Male to female ratio was 2.5:1. The most commonly and severely affected site of the colon was the cecum and proximal colon, although pancolitis was a common finding. At least 60% of the cases had prior or concurrent steroid therapy. The main reported histological changes in the order of frequency were mucosal ulcers, acute and chronic inflammation with abscess formation, eosinophilic granulomatous inflammation, inflammatory polyps and ischemia. All cases had larvae present in the tissue or in the stool.

Conclusions: Histological features of SC seem to significantly overlap with those of UC. A significant number of reported cases are initially diagnosed as UC. Differences between SC and UC albeit subtle are, however, significant enough to lend clues to the correct diagnosis. The most helpful distinguishing features are eosinophil-rich inflammation with eosinophilic abscess formation, microabscess not associated with crypts in the lamina propria, extension to submucosa, and normal intervening mucosa (skip lesion). Presence of larvae in the colonic wall or in the stool is diagnostic. The constellation of clinical history such as steroid therapy, refractory diarrhea, skin rash, pulmonary infiltration, peripheral esinophilia, and endoscopic findings of right-sided colitis can aid in clinico-pathologic diagnosis. In difficult cases, it is prudent to suggest stool and serologic studies for strongyloides, especially in patients who fail to respond to immune suppression therapy for inflammatory bowel diseases.

1226 Use of the Mean Neutrophil Volume Greatly Improves the Laboratory Diagnosis of Bacteremia

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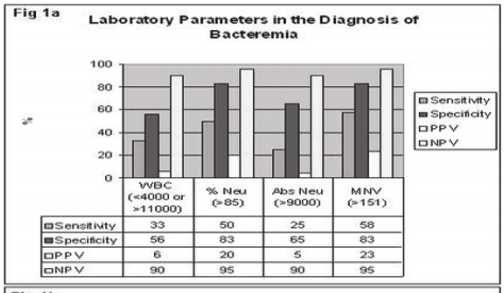
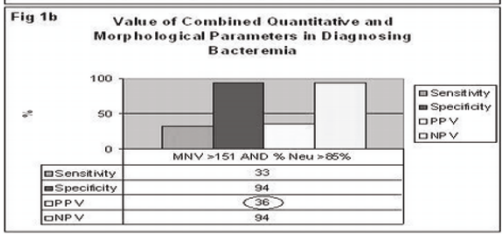
Background: In previous studies (USCAP 2005, Abstract 1205), we have demonstrated that the cell volume of neutrophils (Mean Neutrophil Volume, MNV) increases during acute bacterial infection, and that the sensitivity of this new parameter is superior to that of other commonly used tests, such as the WBC and the neutrophil percentage (%Neu). However, these case-control, proof of concept studies were not meant to fully evaluate the diagnostic potential of the MNV. Since the positive and negative predictive values (PPV / NPV) depend on disease prevalence they could not be calculated. This follow up study was designed to better evaluate the diagnostic value of the MNV, both alone and in combination with other markers for infection, in cases with a high clinical suspicion for this condition.

Design: The study population included all emergency room patients who had a blood culture (BC) ordered on 10 random non-consecutive days (n=150). Data collected included WBC, Absolute Neutrophil Count (Abs Neut), % Neu, and the MNV, tested on blood samples obtained up to 2 days prior to the BC collection. The sensitivity, specificity, PPV and NPV for predicting a positive BC were calculated for each of these parameters. BCs that yielded bacteria likely to be contaminants, such as coagulase negative Staphylococci, were considered negative.

Results: Twelve BCs were reported positive. The value of each of the parameters studied in predicting a positive BC is shown in Fig 1a. Although the MNV showed a lower sensitivity then in previous studies, it still had the best PPV. All parameters had sub-optimal PPVs, reflecting the difficulty of confirming a diagnosis of bacteremia. When we analyzed the MNV and %Neu in combination, we obtained a significant improvement in the PPV, while maintaining the same NPV and Specificity (Fig1b).

Conclusions: The MNV could greatly improve the value of laboratory testing in the diagnosis of bacteremia. It has a better diagnostic performance than other tests traditionally used for this purpose. It is a morphological parameter obtained automatically, at no extra cost, during a regular CBC with differential, and could be most useful if combined with quantitative parameters such as the %Neu.





1227 Identification of Renal Transplant Recipients at Risk for BK Virus Reactivation by Serial Monitoring of Cell Mediated Immunity

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Background: The Cylex ImmuKnow Immune Cell Function Test (ICF) is an FDA cleared assay for detection of cell-mediated immunity in organ transplant recipients. The assay measures the global immune response of CD4 positive T-cells stimulated by phytohemagglutinin. Functional activity of T-cells is expressed in terms of the amount of ATP released, and is characterized as low (≤ 225 ng/ml ATP), moderate (226-524 ng/ml ATP), or high (≥525 ng/ml ATP).

Design: ICF is being used for serial monitoring of cell mediated immunity in renal transplant patients since January 2006. Most patients receive lymphocyte depletion therapy before transplant and Tacrolimus monotherapy post-transplant. We studied 91 paired urine and plasma samples from 52 patients, collected 15-180 days post-transplant, and within 3 days of ICF, for the presence of BK virus DNA using quantitative real time PCR.

Results: BK viremia was found in 3 samples from 2 patients (median 1393, range 1333-1543 copies/ml), with all ICF values falling in the low range, (median 121, range 87-167 ng/ml ATP)**.** BK viruria was detected in 14 samples from 9 patients (median 2.63E+06, range 275.0-6.94E+09 copies/ml) with ICF values of 228 +/- 158 ng/ml ATP. In 74 samples from 41 patients with no BK virus DNA detected in urine or plasma, ICF measurements were 209 +/- 148 ng/ml ATP.

Conclusions: Low functional activity of CD4 positive T-cell lymphocytes, as measured by cross sectional ICF assays, is associated with BK viremia. A similar clear cut relationship is not seen in patients with BK viruria, who may be less immunosuppressed than viremic patients. The potential value of serial ICF measurements to predict viruria needs to be explored further in future studies.

1228 Utility of Immunohistochemistry To Diagnose Cat Scratch Disease

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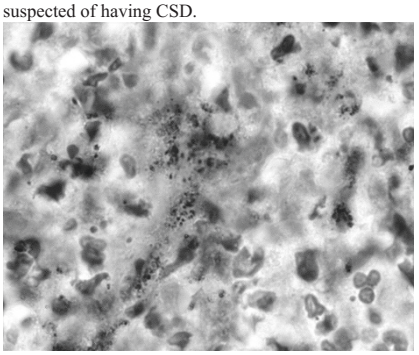
Background: Cat scratch disease (CSD) is largely due to infection with Bartonella henselae (BH). Since BH is difficult to culture, the diagnosis of CSD relies mainly on clinical, serological and/or histological evaluation. The latter requires stellate suppurative granulomas. Silver stains (e.g. Warthin-Starry) for bacteria are cumbersome to perform and hard to interpret. Molecular testing is not readily available. A monoclonal antibody (mAB) to BH has become commercially available. The aim of this study was to evaluate the utility of immunohistochemistry (IHC) to diagnose CSD on routine surgical specimens.

Design: 26 archival formalin-fixed, paraffin-embedded (study) cases of CSD lymphadenitis were investigated. Cases were included if CSD was a clinical consideration and if suppurative granulomas were present. Controls (n=11) included toxoplasmosis (2), sarcoidosis (2), Hodgkin lymphoma (1), dermatopathic lymphadenopathy (1), Rosai-Dorfman disease (1) and non-specific reactive lymphoid hyperplasia (4). 5 micron tissue sections were immunostained with BH mAB at 1:4000, 1:800 and 1:100 dilutions (clone H2A10, Biocare Medical, Concord, CA). PCR was performed following deparaffinization on 50 micron lesional tissue sections. Primer pairs targeted a 153-bp fragment of the 16S rRNA gene present in both BH and B. quintana.

Results: Five (19%) study cases were positive by IHC. Immunoreactive bacteria (see figure) were mainly cocci located predominantly within suppurative granulomas. Nine (35%) study cases were positive by PCR, only one of which was also positive by IHC (at 1:100 dilution only). All controls were negative for both PCR and IHC.

Conclusions: These data show that although the overall diagnostic sensitivity of these tests is low for CSD, PCR is more sensitive than IHC using mAB for BH. IHC positive cases with negative PCR may be attributed to sampling from non-representative lesional tissue. Therefore, we recommend performing PCR on all IHC negative cases suspected of having CSD.



1229 Histopathologic and Immunohistochemical Study of Adrenal Glands in Patients with Fatal Bacterial Infections

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Background: Meningococcemia is the usual bacteria associated with adrenal hemorrhage; however, other bacteria can cause this pathology. The mechanism of adrenal gland hemorrhage is not well understood, in part because visualization of bacteria in the pathologic lesion is difficult. Identification of bacteria using immunohistochemical assays (IHC) could be useful to further understand adrenal gland pathology in patients with fatal infections.

Design: We studied the histopathology of adrenal glands of patients for whom a bacterial infection had been documented by using IHC.

Results: During 1998-2006, 65 cases sent for consultation to the Centers for Disease Control were found to have IHC evidence of a bacterial infection and adrenal glands were included in the tissue submitted for evaluation. The infections diagnosed included *Neisseria meningitidis* in 18 (28%) cases, group A streptococcus (GAS) in 9 (14%), *Rickettsia rickettsii* in 9 (14%), *Streptococcus pneumoniae* in 9 (14%), *Staphylococcus aureus* in 6 (9%), *Ehrlichia sp.* in 3 (5%), *Bacillus anthracis* in 3 (5%), *Leptospira sp*. in 2 (3%), *Clostridium sp*. in 2 (3%), and 1 case of each *Klebsiella sp.*, *Legionella sp*., *Yersinia pestis*, and *Treponema pallidum*. Bacteria were detected in the adrenal of 40 (61%) cases indicating a systemic infection. Adrenal hemorrhage was present in 39 (60%) cases and was associated with bacteria or bacterial antigens in 31 (48%) including 14 with *N. meningitidis*, 4 with *R. rickettsii*, 4 with *S. pneumoniae*, 3 with GAS, 2 with *S. aureus*, and 2 with *B. anthracis*. Nine (14%) cases had evidence of bacteria in the adrenal gland not associated hemorrhage but with inflammatory foci (4 cases), edema (2 cases), congestion (2 cases), or necrosis (1 case). Presence of hemorrhage but without evidence of bacteria in the adrenal was observed in 8 (12%) cases including 2 with *N. meningitidis*, 2 with GAS, 2 with *S. aureus*, 1 with *S. pneumoniae*, and 1 with *Ehrlichia sp*. Seventeen (26%) cases did not show bacteria and the pathology in he adrenal gland was either not significant (13 cases), or mild inflammation (1 case), necrosis (1), and edema (2).

Conclusions: Hemorrhage is the most frequent adrenal gland pathology observed in fatal bacterial infections. Bacterial antigens are frequently seen in adrenal glands with hemorrhage (31 of 39; 79%) and may play a pathogenic role. Although meningococci are the most frequent bacteria associated with adrenal gland pathology, a broad collection of bacteria can cause adrenal lesions.

1230 Which Bug Could It Be? Simultaneous Detection of Multiple Enteric Pathogens Using a Single Multiplex Molecular Assay

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Background: Bacterial enterocolitis is a common worldwide health issue, and a frequent indication for colonoscopy and biopsy. Stool culture has historically been the mainstay of diagnosis, but clinicians frequently treat with antibiotic therapy prior to obtaining cultures, or fail to obtain them at all. Our goal was to design a molecular assay for use on microbiologic specimens and formalin fixed, routinely processed colon biopsies that would simultaneously target seven enteric pathogens using a single multiplex PCR method.

Design: Using both conserved and variable regions of the flagellin gene, we designed an assay to detect seven enteric pathogens through production of species specific base-pair products. Culture isolates for *Salmonella typhi, Salmonella enterica, Campylobacter coli, Campylobacter jejuni, Aeromonas hydrophila, Yersinia enterocolitica,* and *Yersinia pseudotuberculosis* served as positive controls. Reagent blanks and isolates of *Staphylococcus aureus, Mycobacterium tuberculosis* and *M. gordonae* served as negative controls. Thirteen archival colon biopsies from patients with diarrhea and proven bacterial enterocolitis were subjected to multiplex PCR analysis using primers amplifying selected flagellin gene sequences. Fragment analysis was used for product confirmation and control comparison. Final PCR products underwent full sequence analysis.

Results: All 7 enteric pathogen culture isolates serving as positive controls were confirmed using our multiplex assay. Of the archival cases: 5 confirmed as *Campylobacter jejuni*, 3 as *Salmonella enterica*, 1 as *Aeromonas hydrophila*, 1 as *Yersinia pseudotuberculosis*, and 3 as *Y. enterocolitica*, correlating with previous

diagnostic results. No amplificaton was seen within the negative control group. Fragment analysis of PCR products correlated well with the positive control group. Sequence analysis confirmed amplification of targeted specific-specific sequences.
Conclusions: This novel assay, uses PCR and fragment analysis techniques to simultaneously detect seven enteric pathogens in microbiologic specimens and paraffin-embedded, formalin fixed archival biopsy specimens using a single multiplex assay. This assay allows rapid and accurate detection of 7 common enteric pathogens, and is more cost effective than microarray analysis.

1231 Pulmonary Histopathology of Pertussis and Immunohistochemical Localization of *Bordetella pertussis* in Children with Fatal Disease

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Background: Infection with *Bordetella pertussis* results in the clinical syndrome known as pertussis (whooping cough). Despite broad-scale vaccination for this disease, reported cases of pertussis in the U.S. population increased from fewer than 2000 in 1980 to over 25,000 in 2004. *B. pertussis* is fastidious in its culture requirements and a postmortem diagnosis may be difficult to confirm. We describe a novel IHC assay for the detection of *B. pertussis*.
Design: Formalin-fixed, paraffin-embedded tissues from children with laboratory-confirmed, or epidemiologically and clinically suspected, pertussis pneumonia were evaluated with various histochemical stains for bacteria and with an IHC stain using a monoclonal antibody reactive with *B. pertussis* lipooligosaccharide. PCR assays targeting sequences within the IS*481* and pertussis toxin subunit 1 genes were used to amplify and detect DNA of *B. pertussis* from these same tissues.
Results: Tissue specimens from 16 young children with fatal pertussis pneumonia were evaluated; *B. pertussis* was cultured from only 3 of these children. Frequent histopathologic features included intraalveolar hemorrhage, necrotizing bronchiolitis and pneumonia, prominent intravascular collections of leukocytes, and diffuse fibrinous intraalveolar collections of macrophages and neutrophils. Inflammatory infiltrates were absent or minimal in upper airway tissues. Steiner's and Giemsa's stains provided better visualization of bacteria than Gram stains. For all patients, the IHC assay stained bordetella antigens in the cilia of tracheal and bronchial epithelial cells, in inspissated mucus of small airways, and intracellularly in alveolar macrophages. Both *B. pertussis* genomic targets were detected from DNA extracted from formalin-fixed, paraffin-embedded tissues of all patients.
Conclusions: Histopathologic features of pertussis pneumonia provide important presumptive evidence of infection with *B. pertussis* and offer clues to the pathogenesis of fatal disease in young children. IHC and PCR testing provide versatile confirmatory methods for diagnosing fatal pertussis pneumonia in patients for whom conventional methods (e.g., culture) may be negative.

1232 Mycobacterial Spindle Cell Pseudotumor in Immunosuppressed Patients

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Background: Mycobacterial spindle cell pseudotumor is a rare manifestation in immunosuppressed patients with or without HIV infection. Twenty eight patients have been reported in the literature, the majority of whom presented with lymphadenopathy associated with atypical Mycobacteria. A complex host-microbe interaction in immunosuppressed patients is responsible for the formation of spindle cell pseudotumor. Histologically the lesion is composed of proliferative spindle cells, engorged with Mycobacteria and admixed with chronic inflammatory cells. It mimics other spindle cell proliferations including Kaposi's sarcoma, idiopathic inflammatory pseudotumor, malignant fibrous histiocytoma, Hodgkins disease, myofibroblastoma and hemangioendothelioma.
Design: Two recently diagnosed cases are described along with a review of 28 reported cases. Case 1 is a 15 year old male with interferon receptor deficiency, who presented with persistent fever and retroperitoneal and mesenteric lymphadenopathy. Case 2 is a 28 year old male with HIV infection, who presented with persistent fever, splenomegaly and extensive mesenteric lymphadenopathy. Case 1 underwent a lymph node biopsy. Case 2 underwent a splenectomy with a lymph node biopsy.
Results: The biopsied lymph nodes of both patients were diffusely replaced by spindle cells arranged in a loose storiform pattern admixed with lymphocytes and plasma cells. The spleen of patient 2 showed massive spindle cell proliferation with histiocytes, poorly formed granulomas, and acute and chronic inflammatory cells. In each patient Ziehl-Neelsen stain disclosed innumerable acid fast bacilli in the spindle cells and epithelioid histiocytes. Subsequent cultures of blood and bone marrow (patient 1), and blood and spleen (patient 2) grew smooth cream-colored colonies of *Mycobacterium avium-intracellulare* (MAI). Among previously reported cases, the mean age was 36.6 years with a male to female ratio of 12.5. Causes of immunosuppresion include HIV infection(73.3%), organ transplantation, other viral infections and Hodgkins lymphoma. Lymph nodes (58%) are the major organ involved. Mycobacterial cultures revealed MAI in 78% of patients and other microorganisms included *M. tuberculosis*, *M. gordonae* and *M. kansasi.*
Conclusions: Spindle cell pseudotumor is a rare manifestation of mycobacterial infection which mimics other mesenchymal neoplasms in immunosuppressed patients. The accurate diagnosis with identification of organism is of utmost important for appropriate therapeutic approach and prognosis.

1233 Infectious Disease Immunohistochemistry in Placentas from HIV Positive and HIV Negative Patients

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Background: Few studies have evaluated the infectious etiology of placental inflammatory lesions in HIV-positive patients. After reviewing a cohort of placentas from 96 HIV positive and 41 HIV negative patients, we tested a subset of placentas showing chorioamnionitis, funisitis, villitis, and/or deciduitis for etiologic agents known to cause sexually transmitted diseases or fetal infections.
Design: Placentas showing inflammatory changes from 12 HIV positive and 7 HIV negative patients were included. We performed Gram stains and immunohistochemical (IHC) assays for group A and B streptococcus, Neisseria sp., Chlamydia sp., Listeria monocytogenes, Treponema pallidum, Clostridium sp., Toxoplasma gondii, herpes simplex, cytomegalovirus, and HIV p24 antigen in blocks containing placenta, amnion, and umbilical cord. The mothers' infectious disease histories and pregnancy outcomes were compared with IHC results.
Results: An infectious agent was found by IHC in 3 of 7 of HIV negative patients, corresponding to the only cases of intrauterine or immediately postnatal fetal demise. Two cases were positive for Neisseria sp. in the amnion and correlated with maternal history of gonorrhea in one case. These two cases also showed the highest degree of chorioamnionitis of all the HIV negative cases. Group B streptococcus was found on the fetal surface of the amnion and umbilical cord in one case and correlated with presence of gram positive cocci in the same location. In the HIV positive group, one case showed gram positive cocci on fetal membranes; however, organisms were not detected by IHC.
Conclusions: 1. IHC assays for agents known to cause sexually transmitted diseases or fetal infections detected the cause of chorioamnionitis, funisitis, villitis, and/or deciduitis in 16% of cases; these cases of fetal demise also represented the most severe inflammation. 2. In 2 patients the etiologic agent was not suspected previous to IHC, namely Neisseria sp. and group B streptococcus. 3. Although in most cases an etiologic agent could not be found, IHC testing is warranted in cases with fetal demise and severe inflammation. 4. The etiology of inflammation in placentas from HIV positive patients may be due to poorly understood immune mechanisms or untested organisms.

1234 Histopathologic, Immunohistochemical, and Molecular Studies of Adenovirus Infection in Intussusception

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Background: Intussusception is the most common cause of intestinal obstruction in children 3 months to 6 years. The ileocolic intussusception is the most common type although it may occur anywhere in the small intestine and colon. The cause is unknown in most cases but viral infections, especially adenovirus infection, have been implicated in the pathogenesis of intussusception among infants. Surgical reduction is needed when medical treatment fails to revert the intussusception. Surgical samples submitted for pathologic examination usually contain a segment of resected bowel and/or appendix. Culture and other tests for microbial organisms are rarely performed on these samples.
Design: Surgical specimens from 37 infants with intussusception were studied by using histopathologic examination and immunohistochemical (IHC) assay. Polymerase chain reaction (PCR) analysis was performed on cases with positive IHC result. Submitted pathology reports were reviewed and compared with microscopic examination performed at CDC. The antibody used in IHC assay is a monoclonal antibody raised against adenovirus hexon protein. An adenovirus group specific PCR assay targeting the hexon gene and sequencing of amplicons were performed for the confirmation of IHC results and for serotype identification.
Results: Adenovirus was detected in 13 (35%) cases tested by IHC. Smudge cells and viral inclusions were found in the mucosal epithelial cells of these 13 cases. Only two of them were documented to have suspicious viral inclusions by histopathologic examination according to the submitted pathology reports. Immunostaining revealed adenovirus antigens in mucosal epithelium, lamina propria, and lymphoid aggregates. Twelve cases were positive for adenovirus by PCR assay. Sequence analysis of these 12 cases identified adenovirus type 2 in four cases, type 5 in one case, and type 41 in four cases. No distinct sequence could be determined in 3 cases.
Conclusions: Adenovirus is a major etiologic agent causing intussusception among infants. The viral infection usually produces smudge cells and Cowdry type A inclusions in the mucosal epithelial cells. A careful histopathologic evaluation may provide a morphologic evidence to suggest such infection. IHC and PCR assays are useful to confirm the diagnosis. The localization of viral antigens and identification of adenovirus serotypes can also further our understanding in the pathogenesis and epidemiology of intussusception.

1235 Mycobacterial Infections: Histologic Correlation with Molecular Testing and Culture

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Background: Mycobacterial infections are a clinically important diagnosis for patients and from a public health perspective. Therefore, it is important for an accurate and timely diagnosis to ensure proper therapy. In most laboratories, the diagnosis still relies heavily on staining and culture. Staining is limited by its inability to differentiate the many clinically relevant mycobacterial species and culture may require up to 8 weeks for results. Currently, the most rapid method for the identification of mycobacteria is the use of nucleic acid amplification testing.

Design: Results of 50 patients that all utilized culture with acid fast (AF) stained smear, histologic evaluation, and *Mycobacterium tuberculosis* complex (MTb) PCR from the same anatomical site were reviewed. The study period was from July 2003 to August 2006. The archived histologic H&E and AF stained slides were retrospectively reviewed and evaluated for type of inflammation and maximum quantity of AF bacilli (AFB) per 100x field. Available formalin-fixed, paraffin-embedded (FFPE) tissue from patients with at least one positive testing modality were further tested with a real time mycobacterial species PCR assay using meltcurves to distinguish mycobacterial species. A comparison of the methodologies was performed with culture as the gold standard.
Results: Of the 50 patients, 26 (52%) were positive by a least one testing method. On histology, 19 (83%) of these patients demonstrated caseating granulomatous inflammation. By culture, MTb 9) was the most common isolate, followed by *M. avium* complex (7), *M. xenopi* (3), *M. szulgai* (1) and *M. marinum* (1). MTb PCR was positive in 4 cases (sensitivity 44%; specificity 100%). The average AFB quantity for positive and negative MTb PCR was 74 and 1, respectively (p value = 0.0159). The mycobacterial species PCR successfully identified 7/12 nontuberculosis isolates from culture positive patients. Of these, the average AFB quantity for positive and negative mycobacterial species PCR was 85 and 4, respectively (p value = 0.0303).
Conclusions: Nucleic acid amplification testing is a quick and effective method for identifying mycobacterial infection. The utility of PCR on FFPE tissue is of limited value when AFB are scant or absent on histology. However, a PCR assay with the ability to distinguish species provides a greater clinical benefit. Mycobacterial culture remains the gold standard and is necessary for antibiotic sensitivity testing.

1236 Is the Volume of the Added Blood to the Blood Culture Bottles Adequate? A Cross-Sectional Study in a County Hospital

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Background: The optimal volume of the added blood to the BacT/Alert 3D, aerobic and anaerobic blood culture bottle is 8-10 ml.
Design: As part of the quality control study of the microbiology laboratory in our county hospital, we assessed whether the volume of the blood added to the blood culture bottles (BCB) is adequate. 140 consecutive BCB (6 pediatric, 65 aerobic, and 69 anaerobic) were selected. Since one milliliter of blood weighs approximately one gram, the weight of BCBs was used to estimate the volume. Each BCB was weighed with a Miltter AE 100 scale. The weights were recorded in decimals. The weight of the added blood volume was calculated by subtracting the weight of the BCB from a similar unused BCB.
Results: The minimum, maximum, mean and median weight of the BCBs were 0.16, 14.8, 4.53, and 4.37 mg, respectively. **86.43% of the BCBs had less than 6 ml blood, and 2.86% of the BCBs had more than 12 ml blood**.
Conclusions: This study shows that in our county hospital the volume of added blood to BCBs is below the recommended volume of 8-10 ml. Our results suggest that similar studies should be part of the quality control of microbiology laboratories.

1237 Evaluation of Bayer Versant HCV Genotype 2.0 (LiPA 2.0) Assay

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Background: Accurate genotyping of hepatitis C virus (HCV) is essential in the clinical management and monitoring of HCV infections and is also critical in HCV epidemiology studies. Therefore, it is essential to evaluate and choose sensitive and specific HCV genotyping method.
Design: The study evaluated the performance characteristics of the newly developed Versant HCV Genotype 2.0 (LiPA 2.0) assay (Bayer HealthCare, Tarrytown, NJ). The assay uses sequences of both the 5' UTR and core regions to determine HCV genotypes and subgenotypes (1, 1a, 1b; 2, 2a, 2b; 3, 3a, 3b, 3c/d; 4, 4a, 4b, 4a/4c/4d; 5, 5a, 5b; 6, 6a, 6b, 6c, 6d). The LiPA 2.0 assay was compared with Abbott real-time PCR HCV genotyping method which isvcurrently used in our Molecular Diagnostic Laboratory. Discordant genotypes and subgenotypes were examined by sequencing.
Results: A total of 123 HCV positive plasma/serum samples including 114 patient samples and 9 samples from commercial HCV RNA Genotype Performance Panel (PHW202, BBI Diagnostics) were tested by both methods. Twelve of the 114 patient samples were HCV positive by both genotyping methods. While Versant LiPA 2.0 successfully genotyped these 12 samples as 1a (2 cases), 1b (8 cases), and 3a (2 cases), Abbott Real time PCR method failed to genotype these samples (reported as indeterminate). Versant LiPA 2.0 assay correctly genotyped all samples in the PHW202 panel while Abbott assay failed to genotype the 1b sample (typed as indeterminate). These "indeterminate" HCV samples accounted for the great majority of the discordance (14/18 = 77.8%) between these two methods. The assay sensitivity was examined using series dilutions of two previously genotyped 1a and 1b samples by both methods. Versant LiPA 2.0 genotyped both subtypes at 1600 IU/mL, whereas Abbott assay genotyped 1a at 1600 IU/mL, but failed to genotype 1b even when the HCV viral load was much higher (e.g., 5,000 IU/mL,).
Conclusions: Compare with Abbott real-time HCV genotype assay, Versant LiPA 2.0 showed higher degree of genotyping sensitivity for subtyping 1b. To our knowledge, this is the first time the sensitivity level of Versant LiPA 2.0 assay has been evaluated.

Kidney

1238 Lymphoid Cell Proliferation in Renal Transplants. Biologic and Diagnostic Implications

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Background: The initiation site of alloimmunity during renal transplant rejection remains controversial. It is not clear whether the alloreaction develops in peripheral lymphoid organs with the effector cells being subsequently recruited to the target organs, or the entire process of alloreaction including antigen presentation, maturation and proliferation of the effector cells can happen within the transplanted kidneys. Evaluation of the proliferation of inflammatory cells within the transplanted kidneys and the involved cell types may provide further insight and may aid in the diagnosis of acute cellular rejection (ACR).
Design: Interstitial inflammatory cell proliferation was evaluated by immunostain for MIB-1 in 129 kidney specimens. Double immunostain was performed to determine the cell types, i.e., T cells , B cells, or macrophages, that underwent proliferation The results were correlated with clinical features.
Results: The percentage of inflammatory cells in proliferation was 25.7 ± 5.4 in ACR (n = 24), which was significantly higher than in normal kidney (0.4 ± 0.2, n = 8), acute tubular necrosis (1.2 ± 0.5, n = 8), chronic allograft nephropathy (CAN) (2.4 ± 0.6, n=20), and native kidneys with diverse diseases (n = 63, 9.2 ± 2.3). It was, however, comparable to that in CAN with significant intertitial inflammation (20.6 ± 3.9, n= 16). The percentage of T cells in proliferation was 16.1 ± 2.1 and 18.9 ± 2.0 in ACR with or without CAN, whereas those for B cells or macrophages were low (< 1.7%), regardless of diagnostic categories. All cases diagnosed as ACR in conjunction with a high rate of MIB-1 + inflammatory cells, including one with superimposing BK virus nephropathy and one with "borderline" features; and 9 out of 12 cases with CAN and significant interstitial inflammation in which superimposing ACR was diagnosed, in part due to a high rate of MIB-1 + inflammatory cells, responded well to antirejection therapy.
Conclusions: Proliferation of interstitial inflammatory cells can be readily detectable in renal tranplants with ACR and this involves predominantly T cells. These observations not only provide additional support for the controversial concept of *in situ* alloimmunization, but may also facilitate the diagnosis of ACR in problematic cases.

1239 Alpha Heavy Chain Deposition Disease: A Comparison of Its Clinicopathological Characteristics with Gamma and Mu Heavy Chain Deposition Disease

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Background: Heavy chain deposition disease (HCDD) is a rare monoclonal immunoglobulin disorder characterized by the production and deposition of immunoglobulin heavy chains without a light chain. The predominance of gamma HCDD with only two cases of alpha HCDD reported thus far, prompted this study in which we describe two additional unusual forms of alpha HCDD and summarize and compare the clinicopathological characteristics of all identified cases in the literature.
Design: The clinical presentation, laboratory data, and pathology of 24 patients with HCDD, inclusive of two of our cases were compiled.
Results: The results are summarized in the table below. Gamma is the predominant heavy chain subset. Most patients present in the fourth to fifth decade with impaired renal function, proteinuria, and hypertension. Hypocomplementemia is exclusive to the gamma subset. Definitive association with a plasma cell dyscrasia is seen in all cases of alpha HCDD and only a small proportion of Gamma HCDD. The patterns of injury are predominantly nodular sclerosing glomerulopathy, with a dominant crescentic pattern of injury in the alpha HCDD and a rare endocapillary proliferative pattern in gamma HCDD. Extra-renal deposition is noted, with two cases of cutis laxa, single case of deposition in liver and thyroid, and another with skeletal muscle involvement. We documented unique binding of alpha heavy chains to elastic fibers in the skin in one of our cases.
Conclusions: Alpha HCDD, although less frequent among the HCDD, is often characterized by a crescentic pattern of injury and an associated plasma cell dyscrasia. A unique complication in few cases of HCDD is cutis laxa, possibly a consequence of active inflammation and elastolysis in response to the binding of the heavy chain to elastic fibers.

Summary of clinicopathological characteristics

Clinical characteristics	Alpha HCDD (n=4)	Gamma HCDD (n=19)	Mu HCDD (n=1)
Age range (average)	29-78(55)	35-73(54.3)	63
M:F	3:1	2:3	0:1
Cutis laxa	1/4	0	0
Urticarial vasculitis	1/4	0	0
Hypertension	4/4	16/19	1/1
Nephrotic range	3/4	13/19	1/1
Hypocomplementemia	0	10/19	0
Myeloma	4/4	3/19	0
Hepatitis C positivity	1/4	5/19	0
Nodular sclerosis	3/4	17/19	1/4
Crescents	3/4	3/19	0
Extraskeletal deposition	skin	skin, muscle, thyroid,liver	0