1176 FISH Suggests That MIB-1 Labeling Index Is Not a Reliable Distinguisher of Atypical Burkitt Lymphoma from Diffuse Large B-Cell Lymphoma

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Background: Atypical Burkitt lymphoma (ABL) is defined as a highly aggressive B-cell neoplasm of predominantly medium-sized cells with non-classic Burkitt morphology, such as increased atypia, a nearly 100% growth fraction, and consistent translocations involving the c-*myc* gene. Because of its pleomorphism, ABL is often confused with diffuse large B-cell lymphoma (DLBCL), which has abundant cytoplasm, vesicular nuclei and small or prominent nucleoli, and not infrequently a similarly high proliferation rate. Although the distinction of these two entities has significant clinical implications, the differential diagnosis is difficult in some cases.

Design: We have selected 7 challenging cases of B-cell lymphoma with atypical morphology. Six cases were nodal and one was derived from bone marrow. Patients ranged in age from 19 to 71 years (median age of 62 years), and included 3 males and 4 females. ABL and DLBCL was the differential diagnosis in each case. We studied these cases using immunohistochemistry, flow cytometry and fluorescence in situ hybridization (FISH) using c-*myc* break-apart and *IgH/BCL2* dual fusion probe sets.

Results: H&E sections showed that the tumor cells varied from medium to large in size with slightly clumped to vesicular nuclei and occasional prominent nucleoli. None of the cases showed a classic Burkitt morphology. All the cases were CD20+ except for case 3 that was CD20–/CD79a+. Proliferation indices as assessed by MIB-1 stain varied from 50% to 100%. A c-*myc* rearrangement was detected in 5 cases with labeling indices <90% whereas it was absent in 2 others with >90% labeling. In addition, a t(14;18) was additionally detected in 2 cases with a c-*myc* translocation. ABL was diagnosed in 5 cases with a c-*myc* translocation, and DLBCL was diagnosed in 2 cases with no c-*myc* translocation.

Conclusions: We conclude that the growth fraction is neither absolutely specific for the diagnosis of ABL, nor does it entirely exclude the possibility of DLBCL. The c-*myc* translocations are more reliable in resolving the diagnosis in cases with a confusing morphology. The significance of ABL cases harboring both c-*myc* rearrangement and a t(14;18) is unclear and this subgroup needs to be further defined.

1177 B-Cell Chronic Lymphocytic Leukemias with p53 Deletion Are Highly Resistant to Fludarabine *In Vitro*

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Background: Individual patients with B-CLL demonstrate variable responses to standard induction and salvage therapeutic regimens. It would be highly desirable to develop a predictable and reproducible laboratory diagnostic strategy that guides the selection of appropriate drugs and/ or regimens based on the drug sensitivity and resistance profiles of leukemic cells for individual patients.

Design: A study was designed to investigate the differences of in vitro drug sensitivity profiles of leukemic cells with different cytogenetic abnormalities from CLL patients. CLL cells from 43 patients were incubated *in vitro* with four commonly used chemotherapeutic agents (fludarabine, chlorambucil, cladribine or prednisolone). Multiparameter flow cytometry was utilized to determine the decrease in leukemic cell viability after drug exposure. The *in vitro* drug sensitvity profile were correlated with the cytogenetic abnormalities and clinical responses retrospectively.

Results: The highest *in vitro* resistance to fludarabine, was seen in all seven cases of B-CLL cells with deletions of p53, a cytogenetic abnormality associated with poor clinical outcome and poor clinical response. A majority of cases highly resistant to fludarabine were also resistent with chlorambucil and cladribine, but not to prednisolone. In CLL cases without p53 deletion, a marked variability in drug sensitivity was observed *in vitro* but no significant difference was detected among cases with normal cytogenetics (n=13), ATM deletion (n=4), trisomy 12 (n=3), or 13q deletion (n=7).

Conclusions: Our findings provide direct evidence of cellular resistance to fludarabine in CLL associated with p53 deletion, confirming prior clinical observations. *In vitro* drug sensitivity assay with routine clinical specimens containing leukemic cells admixed with normal cells. This method may prove useful in guiding choices for therapy for CLL patients based on the drug sensitivity profile of leukemic cells in individuals.

1178 Utility of Flow Cytometry in Detecting CNS Involvement by Hematopoetic Malignancies

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Background: Flow cytometry (FC) and morphology (M) are techniques implemented in the evaluation of cerebrospinal fluid (CSF). The purpose of the study was to assess the utilization and the diagnostic accuracy of FC in CSF evaluation in a large number of samples and to determine when FC would be a useful adjunct to routine M examination. Independent FC and M data were compared, with emphasis given to total cell count (TCC).

Design: Reports from 313 cases of CSF analyzed by 4-color FC were stratified as follows: insufficient hematopoetic cells, no abnormal population, and abnormal population detected. TCC was available for 102 of these cases. Clinical history was obtained for 227 cases from the past two years. Of these, there were 161 cases with independent M and FC reports of the same specimen, in which these two reports were signed-out without knowledge of the other report. Discordant reports were correlated with clinical history and TCC. Sixty-six of the 227 were eliminated as the reports were not independent or no M evaluation was performed.

Results: At this institution, FC analysis of CSF has increased more than 100% (86 samples in 2003, 109 in 2004, projected 177 in 2005). However, insufficient hematopoetic cells (26-31%) and abnormal populations detected (20-22%) have remained constant. Of those with an abnormal population, 48% had a TCC of less than 5000, including cases with less than 500 cells. Of the 227 cases, 80% had a history of a hematological malignancy. Of those with an abnormal population by FC, 6% (n=3) had no history of a hematopoetic disorder. Analysis of 161 cases by independent FC and M revealed 87% (n=140) concordance. Of these 161, over 50% of the cases in which FC detected an abnormal population not seen by M, occurred in specimens with a TCC of less than 5000. Abnormal populations were detected by M and not noted by FC in 2% (n=3). Abnormal populations were detected by FC but reported as negative by M in 11% (n=18). Interestingly, abnormal populations were detected by FC and not M in 62% (n=8) of mature T-cell neoplasms.

Conclusions: This comprehensive study revealed that FC can detect abnormal populations not evident by M in samples with low TCC. Thus, limiting the usage of FC based on TCC is not recommended. FC in conjunction with M examination yields enhanced detection of abnormal cellular populations in CSF. FC analysis of CSF in patients without history of hematological malignancy can detect lymphoproliferative disorders. Analysis of data at this institution reveals that the increased usage of FC in the analysis of CSF is warranted.

Infections

1179 Correlation of Cytokine Expression, Histologic Findings, and Clinical Outcome in the Placenta

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Background: Histological examination of placentas with in utero infections often lacks (on H&E sections) the typical lymphocytic or neutrophilic response associated with infection in other organs, and more fequently shows non-specific changes. Placental macrophages and trophoblasts are capable of producing many cytokines. These cytokines could be used as a marker for prenatal infections.

Design: We examined the histologic findings in 90 placentas and correlated this with the expression of cytokines (macrophage inflammatory protein alpha (MIP), IL-8, and tumor necrosis factor alpha (TNF)) as determined by immunohistochemistry, and clinical outcome. Of the 90 placentas, 40 were from either stillbirths (20) or from cases of severe morbidity (APGARs <5/5 with severe sequela) in which an infectious agent was identified (most cases were bacterial infection or coxsackie virus infection). The other 50 were the controls, all with APGARs >8/8 and no clinical problems. The 50 controls included cases of diabetes mellitus (10) and preeclampsia (10). Increased expression of a cytokine was defined by noting at least 5 positive cells per placenta section.

Results: Of the 50 controls, increased expression of a cytokine was evident in only 2 cases (4%). Of the 40 cases of severe morbidity/mortality of infectious cause, at least one cytokine was increased in 40/40 cases. Most cells expressing TNF were trophoblasts or macrophages, whereas MIP and IL-8 expression was noted mostly in macrophages. In comparison, we studied 20 cases of known neonatal morbidity/mortality that was not infectious (eg abruption, ruptured uterus, prolapsed cord). In 2/20 (10%) of these cases, there was increased expression of a cytokine. No histologic finding correlated with cytokine expression. Further, there was no correlation between the number of CD68 or CD45 positive cells with cytokine expression.

Conclusions: Marked increase in cytokine expression is seen in placentas with in utero infections associated with severe morbility/mortality. This marked increase in MIP, TNF, and/or IL-8 appears to be a specific and sensitive marker of severe in utero infection, as it was rarely noted in controls.

1180 Cytomegalovirus Gastrointestinal Disease in the Elderly

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Background: CMV is the most common viral cause of GI morbidity in immunosuppressed patients with AIDS and transplantation. It is also seen in refractory IBD patients. We have encountered CMV infections in the elderly without significant history. It is the objective of this study to determine the relative percentage of affected elderly patients diagnosed with CMV and to analyze clinical information for the presence of known risk factors.

Design: We identified 32 biopsies in 26 patients diagnosed with CMV infection from January 1998- March 2004 in two community hospitals, Advocate Lutheran General Hospital and Advocate Illinois Masonic Medical Center. Tissue blocks and slides were all available, retrieved, reviewed and stained for AFB (modified Kinyoun's), fungi (GMS), and immunostained for HSV I/II, CMV/clone DDG9/CCH2 and Adenovirus/clone 20/11/2/6 (Ventana Medical Systems, Inc., Tucson, AR). Cells were positive only if nuclear staining were present. Clinical and endoscopic data were collected if available.

Results: Seven of 26 patients (27%), 4 males and 3 females, were elderly. The clinical features, endocopic findings, biopsy site and follow up data are presented in Table 1. All cases showed varying degrees of acute and chronic inflammation, granulation tissue and focal ulceration with CMV inclusions. They were in endothelial cells in 6, epithelial cells in 2, and stromal cells in 4 cases. CMV IHC were positive in 4 cases. None had co-infection with other viral, mycobacterial, fungal and protozoal pathogens by H&E, special and IHC.

Conclusions: Our findings demonstrate that a significant number of biopsies with CMV GI disease occur in the elderly, most with history of immunosuppression. A significant minority will lack typical risk factors for CMV infection. We conclude that CMV should be included in the differential diagnosis of elderly patients with unexplained GI inflammation.

Table 1. The clinical features of seven elderly patients with CMV gastrointestinal infection.

Age	Sex	Symptoms	Endoscopy	Sites	Follow-up
73	M	odynophagia	ulcerations	esophagus	LTF (NSCLC)
77	M	chronic diarrhea	erythema	colon	LTF (NSCLC)
78	F	screening colonoscopy	pseudo-polyp	transverse colon	LTF (aortic stenosis)
67	M	weight loss	mucosal irregularity	esophagus	D (AIDS)
72	F	abdominal pain	erythema	stomach	AW (DM type II)
77	M	epigastric pain	erythema	stomach	AW (DM type II)
73	F	rectal bleeding	erythema	rectum	AW (CLL)

Abbreviations: AW, alive and well; LTF, lost to follow-up; D, dead; NSCLC, non-small cell lung carcinoma

1181 Microbial Co-Infections with Biopsy-Proven CMV GI Disease

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Background: CMV is a beta herpes virus that infects only human cells. The GI tract is a major site of infection usually affected immunosuppressed individuals. In this setting, multiple infections may occur. It is the objective of this study to determine the relative incidence of microbial co-infections of biopsy-proven CMV GI diseases in a contemporary community hospital based study.

Design: GI biopsies with CMV infection from January 1998- March 2004 in two Chicago-wide community hospitals were identified through a SNOMED COPATH search. Tissue blocks and slides were all available, retrieved, reviewed and stained for AFB (modified Kinyoun's), fungi (GMS), HSV I/II, CMV/clone DDG9/CCH2 and Adenovirus/clone 20/11/2/6 (Ventana Medical Systems, Inc., Tucson, AR). IHC for adenovirus and HSV was interpreted as positive only if nuclear staining was present. Clinical/ endoscopic information was collected if available.

Results: Thirty-two biopsies from 26 patients were diagnosed with CMV. The median age was 51, and the mean was 52 (range: 20-78). The male to female ratio was 1.6:1. Eleven had AIDS/HIV+. Two had ulcerative colitis. Three had malignancies (CLL and non-small cell lung cancer(2)). Biopsy sites were as follows: esophagus, 8; stomach, 6; stomach and duodenum, 1; ileum, 1; colon, 12; and rectum, 4. All biopsies were diagnosed with CMV based on distinctive cytomegaly with inclusions which were seen in endothelium in all cases, stromal cells in 15 cases and epithelial cells in 8. Only 23 cases were reactive with anti-CMV. Four of 32 biopsies had infections in addition to CMV. Two esophageal biopsies exhibited distinctive HSV inclusions. IHC for anti-HSV was positive in one case. Two biopsies were positive for cryptosporidiosis, and both were seen on GMS stains. The clinical features of these 4 cases are presented in Table 1. All were HIV+.

Conclusions: Our findings showed that a significant minority of HIV/AIDS patients with CMV GIT disease harbored other opportunistic infections. The presence of CMV inclusions on biopsy specimens warrants a search for microbial co-infections.

Table 1. The clinical features of four patients with co-infections with CMV GI infection.

Age/sex	Symptoms	Endoscopic signs	Biopsy site	Co-infection	Follow-up
39 F	odynophagia	ulceration	esophagus	HSV	D (Disseminated MAC)
31 M	odynophagia	loss of vascularity	esophagus	HSV	LTF
49 F	chronic diarrhea	ulceration	sigmoid colon	cryptosporidiosis	LTF
37 M	chronic diarrhea	ulceration	gastric/ duodenal	cryptosporidiosis	AW

Abbreviations: D, dead; LTF, loss to follow-up; AW, alive and well.

1182 Association between Epstein-Barr Virus (EBV) and Composite Lymphomas: An *In Situ* Hybridization Study Using EBV-Encoded RNA (EBER) Probes

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Background: Composite lymphomas are lymphoid malignancies in which at least two distinct lymphoma subtypes coexist within the same lymph node or in an extranodal site. These lymphomas are rare with only isolated cases or small series reported previously. In addition, prior studies have shown that EBV is associated with up to 50% of the classical Hodgkin lymphomas. Furthermore, the virus is shown to be present in only neoplastic cells of Hodgkin lymphoma supporting the hypothesis that the virus plays a role in pathogenesis of Hodgkin lymphoma. The possible role of EBV and its association with composite lymphomas have not been previously investigated. We studied six cases of composite lymphomas, all with a component of Hodgkin to analyze the possible role of the virus in these rare subtypes of lymphoid malignancies.

Design: From the files of the Department of Pathology at the University of Iowa Hospitals and Clinics, we identified eleven cases with a prior diagnosis of composite lymphoma. Among these, six cases were identified in which classical Hodgkin lymphoma accounted for one of the two components of composite lymphoma and only these cases were included in the study. The second component included: 3 diffuse large B-cell lymphoma (DLBCL) and 3 follicular lymphoma (FL). *In situ* hybridization was performed using paraffin-embedded tissue sections and EBV-encoded RNA (EBER) probes.

Results: The patients' age ranged from 30 to 83 years with a median age of 50 years. One out of 3 composite lymphomas with a component of DLBCL revealed EBV-infected cells in both Hodgkin lymphoma and DLBCL. The EBV was also detected in only Hodgkin component of one out of 3 cases with FL components. The expression of EBV in the positive cases was exclusively limited to the neoplastic cells. None of the follicular lymphoma components expressed EBV.

Conclusions: The unusual and rare cases of composite lymphomas provide an opportunity to study the molecular pathogenesis of seemingly unrelated processes as well as possible role of EBV which is known to be associated with some subtypes of lymphomas. Although the number of cases is low, the results of our study may suggest that in EBV-positive composite lymphomas, the EBV may play a role in pathogenesis if the non-Hodgkin lymphoma component is a high-grade one such as DLBCL. The EBV is unlikely to be associated with those composite lymphomas in which the non-Hodgkin lymphoma component is a low-grade one such as follicular lymphoma.

1183 Downregulation of ICAM-1 Gene Expression in Lymphoid Tissue of Chronic HIV-1 Infected Patients after HAAR

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Background: Viral replication and reservoir of HIV-1 occur in lymphoid tissue. HIV-1 infects follicular dendritic cells (FDC) and lymphoid cells. In vitro studies have demonstrated that mature HIV-1 particles are studded with ICAM-1 that is crucial for the HIV immune response. This protein is expressed in endothelial cells and is responsible for the adhesion and recruitment of leucocytes in lymphoid tissues. Moreover, ICAM-1 plays and important role in the interaction between HIV-1 and FDC cells which are responsible for transmitting the infection to CD4+ T cells. We studied ICAM-1 mRNA and protein expression in tonsil tissue from HIV patients and the correlation with tissular viral load.

Design: Sixteen patients with chronic asymptomatic HIV-1 infection and CD4+ lymphocyte > 500 cells x 10^6 /L were eligible for tonsillar biopsies before initiating antiretroviral therapy (HAART) and after 12 months of treatment. The lymphoid tissue viral load was determined by PCR. Tissue sections were investigated for ICAM-1(follicular and endothelial) protein expression by immunohistochemistry and evaluated in three grades (negative, 1+ and 2+). ICAM-1 mRNA from frozen tissue was measured before and after HAART in 6/16 patients using quantitative, Taqman® RT-PCR.

Results: Initial biosies before HAART showed a high tissue viral load and a high protein expression (2+) of ICAM-1 in FDC in 10/16 cases and in endothelial cells in 13/16 cases. After HAART the viral load decreased significantly. The ICAM-1 protein expression was also lower with negative or weak staining (0 or 1+) in FDC (14/16) and in endothelial cells (10/16). ICAM-1 mRNA also decreased significantly in all cases. ICAM mRNA expression correlated with protein expression and viral load.

Conclusions: ICAM-1 protein expression in FDC and endothelial cells and ICAM-1 mRNA decreased after HAART in patientes with chronic HIV infection. Low levels of viral load correlated with low levels of ICAM-1 mRNA and protein expression suggesting a transcriptional up regulation of ICAM-1 gene expression induced by HIV-1 in host cells.

1184 Immunohistochemical Assay for *Streptococcus pneumoniae* in Autopsy Cases: Can Sensitivity and Specificity Be Obtained?

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Background: *Streptococcus pneumoniae* is the most frequent cause of community-acquired pneumonia and meningitis. *S. pneumoniae* cultures should be correlated with clinico-pathologic features since the bacteria are found in respiratory secretions of 5% to 70% of normal individuals or can test negative for infected individuals receiving antibiotics. Immunohistochemical (IHC) assays identify and localize bacterial antigens in the inflammatory reaction.

Design: An indirect IHC assay using a polyclonal anti- *S. pneumoniae* antibody was optimized for formalin-fixed, paraffin-embedded (FFPE) samples. The antibody reacted against FFPE cultures of *S. pneumoniae* but did not react with FFPE cultures of *S. pyogenes*, group B *Streptococcus*, *S. aureus*, and other bacteria. The IHC assay was applied to autopsy samples from 30 patients selected from a medical examiner surveillance system and from 12 consultation cases that had pre or post-mortem cultures. IHC sections were reviewed in a non-blinded manner and compared with culture results to determine the sensitivity and specificity.

Results: Positive *S. pneumoniae* IHC staining was present in samples from 26 patients, 18 of whom had *S. pneumoniae* culture results including 15 with pneumonia, 2 with meningitis, and 1 with septicemia, while 8 patients (7 with pneumonia and 1 with meningitis) had cultures that grew mixed bacteria (4 cases) or had no growth (4). *S. pneumoniae* antigens were found in areas of inflammation (lung or meninges) or inside inflammatory cells in blood vessels or sinusoids. *S. pneumoniae* IHC was negative in 7 cases that grew *S. pyogenes*, 5 that grew *S. aureus*, and 4 that grew *H. influenzae*. The IHC assay showed a sensitivity of 100%, specificity of 67%, positive predictive value of 69%, and negative predictive value of 100%.

Conclusions: Specificity and positive predictive value results were affected by limitations imposed by using cultures as the reference standard and the 8 patients without culture confirmation for *S. pneumoniae* most likely represent true cases detected by the IHC assay. Complementary tests that can confirm *S. pneumoniae* in FFPE samples of cases for which cultures are not contributory need to be developed. IHC assay for *S. pneumoniae* is an important diagnostic method that can be applied to FFPE autopsy samples with excellent sensitivity and negative predictive value.

1185 *Lecythophora mutabilis* Endocarditis: An Unusual Cause of Prosthetic Aortic Valve Endocarditis

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Background: Fungal endocarditis may result from several predisposing factors such as cardiac surgical treatment, prolonged hospitalization, indwelling central catheters etc. It accounts for roughly 15% of all prosthetic valve endocarditis. We present a rare case of prosthetic aortic valve infection with *Lecythophora mutabilis* and discuss the difficulties in making an accurate diagnosis.

Design: A 58 year old diabetic male, with multiple medical problems, presented with progressive dyspnea due to critical aortic valve stenosis. He underwent aortic valve

replacement with a Carpentier Edwards Pericardia Magna tissue valve, as well as a two-vessel coronary artery bypass graft (CABG). Four months after surgery an internal defibrillator device was placed but had to be removed urgently due to MRSA infection with bacteremia. Approximately five months after this episode, he presented with intermittent fever, chest pain and dyspnea at rest. Blood cultures remained negative on multiple occasions. A transthoracic echocardiogram (TTE) showed poor visualization of the aortic valve and a transesophageal echocardiogram (TEE) demonstrated a large mass originating from the aortic valve prosthesis. This measured 4-5 cm in greatest dimension and led to removal of his prosthesis and placement of a pericardial tissue valve. Gram stain revealed 1+ polymorphonuclear leucocytes and branching hyphae consistent with a fungal infection. Surgically removed tissue was submitted for histopathologic review as well as microbiological analysis.

Results: On routine histologic examination the vegetation showed septate, hyphal fragments with bulbous swelling and branching reminiscent of *Aspergillus* or *Pseudallescheria* species. Microbiologic cultures on blood, chocolate and EMB agar showed no growth. Low waxy colonies with few aerial hyphae were seen on lactophenol cotton blue (LCB) scotch tape and Sabouraud's medium and acquired a salmon color. BHI and SAB tubes showed low, waxy, yellow-beige colonies with central brown pigmentation. However, definitive identification of the isolate was not possible using phenotypic methods alone. Genotypic identification was necessary to confirm the identity of the fungus as *Lecythophora mutabilis*. The patient was treated with dual anti-fungal therapy and is being maintained on prophylaxis with voriconazole.

Conclusions: Lecythophora mutabilis should enter the differential diagnosis of fungal infections that may mimic *Aspergillus* or *Pseudallescheria* species on morphology and colonize prosthetic valves in susceptible patients.

1186 Detection of Human Herpesviruses in Kikuchi-Fujimoto Lymphadenitis

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Background: Kikuchi-Fujimoto disease (KFD) is a subacute usually self-limited inflammatory disorder of unknown cause most commonly seen in young women who present with mild fever and cervical lymphadenitis, sometimes with sore throat and skin rash. Features of the clinical presentation and histopathology suggest an infectious, likely viral, etiology. Although investigations to identify KFD-associated infectious agents have yielded mixed results, the most commonly suspected infectious agents are the human herpesviruses EBV, HHV-6, HHV-7, and HHV-8.

Design: We have screened DNA extracted from FFPE lymph node biopsy tissues of 20 cases of histologically-confirmed KD from the U.K. and Saudi Arabia for the presence of all eight known human herpesviruses by two techniques, i.e. nested pan-herpes PCR using a set of degenerate consensus primers followed by dot blot hybridization, and real time quantitative multiplex PCR with Taqman probes, supplemented in the case of EBV with nested PCR for EBNA1. DNA quality from samples yielding negative results was determined by PCR for the housekeeping gene GAPDH. Colorimetric in-situ hybridization for detection of intranuclear EBER1/EBER2 RNA transcripts was performed on unstained paraffin-embedded sections to directly visualize EBV-infected cells within lymph node tissues.

Results: Lymph node tissues from 10 of 20 cases (50%) of KFD were EBV positive using a highly sensitive nested EBNA1 PCR method. 4 of 8 available EBNA1+ cases were also EBV positive by real time PCR. In 2 EBV PCR+ cases, a second herpesvirus was detected by real time PCR, CMV in one case and HHV-7 in another. In no case was another herpesvirus detected in the absence of EBV. Pan-herpes consensus PCR and real time herpesvirus PCR for all other human herpesviruses (HSV, VZV, HHV-6, HHV-8) was negative in all cases. Scattered EBER ISH+ cells were identified in 7 of 10 EBNA1 PCR+ cases. Remarkably, EBER positivity was confined to small lymphoid cells with apoptotic features within necrotizing regions.

Conclusions: The presence of EBV DNA in 10 of 20 (50%) cases of KFD lymphadenitis supports the notion that EBV infection may play a role in the pathogenesis of at least some cases of this peculiar inflammatory disease. The detection of EBV RNA (by in-situ hybridization) in small apoptotic lymphoid cells within necrotizing regions of the lymph nodes suggests that the necrotizing lesions characteristic of KFD are due to a vigorous necrotizing inflammatory response to EBV-infected lymphoid cells.

1187 Actinomyces Infection Mimicking Recurrent Squamous Cell Carcinoma in Treated Head and Neck Cancer

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Background: *Actinmycosis* is an uncommon but treatable complication following multi-modality treatment for squamous cell carcinoma of the upper aero-digestive tract. The infection mimicks recurrent carcinoma clinically and therefore may not be biopsied and when biopseid the pathologist my not identify or specify the type of organism.

Design: We present the histologic features of destructive *Actinomycosis* infection identified by the pathologist on morphological features in four patients following radiation, chemotherapy and/or surgery. The early diagnosis prompted an immediate antibiotic treatment. In all patients symptoms improved dramatically and rapidly after penicillin therapy. Hemotoxaline and eosin stained sections with gram stain. Retrospective chart review of four cases.

Results: Complications directly attributed to *Actinomycosis* infection includes pharyngeal edema, severe laryngeal edema, neck mass and osteonecrosis of bone. Diagnosis was made by biopsy showing numerous bacterial colonies destroying the bone trabeculea (fig-1) which was undergoing remodeling. Gram stain highlights the gram positive organisms (fig-2) showing gram positive beaded branching bacteria. The antibiotic of choice is penicillin with the length and type of the treatement dependent on the site of infection and the severity of the symptomes. At the conclusion of treatment, all symptomes resolved, including mucosal coverage of the previously exposed bone.

Conclusions: Actinomycosis is a potential complication in patients following multi-modality treatment for squamous cell carcimoma of the upper aero-digestive tract. The pathologist has an immportent role in identifiying and informing the clinician at a timely manner. Hence treatment of infeciton is vital to hasten the resolution of symptoms.

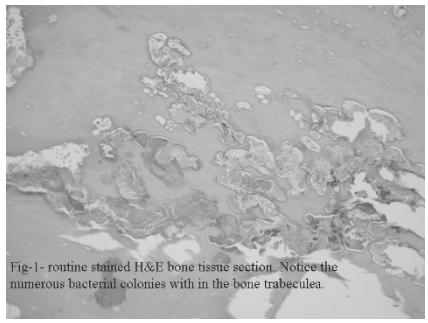

Fig-1- routine stained H&E bone tissue section. Notice the numerous bacterial colonies with in the bone trabeculea.

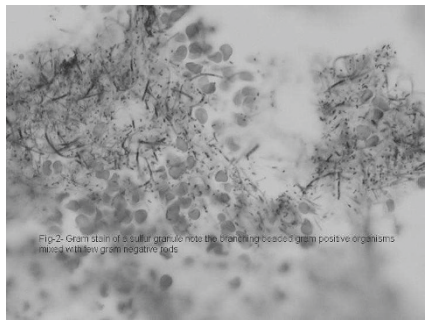

Fig-2- Gram stain of a sulfur granule note the branching beaded gram positive organisms mixed with few gram negative rods

1188 Pathologic Diagnoses of Cutaneous Diseases Clinically Mimicking Leishmaniasis

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Background: Military and civilian personnel deployed to Southwest or Central Asia (Afghanistan, Iraq, and Kuwait) or other endemic areas are at risk for cutaneous leishmaniasis (CL), a sand fly-borne parasitic infection. However, a significant number of patients presenting with persistent skin lesions mimicking CL actually have other diseases.

Design: During 2002 to 2005, we examined histology or cytology specimens from 1018 patients suspected of having CL. Cytology specimens had been prepared by making touch preps from biopsies or by making smears from scrapings of lesions. Methods for confirming CL included microscopic identification of the parasite or polymerase chain reaction (pcr) or both. We did not make a diagnosis of CL in 316 (31%) of them. We reviewed the specimens from these patients in order to characterize those in which there was a specific diagnosis other than CL. We eliminated from the study 202 patients in whom the histologic features were consistent with CL (i.e., ulceration or granulomatous dermatitis), but in whom no parasites were identified by microscopy or pcr.

Results: We made 126 diagnoses on the remaining 114 patients (12 patients had 2 diagnoses). Thirty-six of these diagnoses were only descriptive and nonspecific, including keratosis, acanthosis, folliculitis, and epidermal hyperplasia. Forty-three were inflammatory conditions, such as various forms of dermatitis, arthropod reactions, lichen planus, lichen simplex chronicus, prurigo nodularis, lupus, vesiculobullous disease and sarcoidosis. Twenty six were viral, bacterial or fungal infections. Nineteen were neoplastic including basal cell carcinoma, various melanocytic lesions, dermatofibroma, eccrine acrospiroma, and fibrous histiocytoma. There were 2 cases of vascular disease.

Conclusions: Health-care providers should certainly consider CL as the cause of non-healing skin lesions in persons who have been deployed to endemic areas. However, in a significant number of cases, in which CL cannot be confirmed by light microscopy or pcr, the diagnosis is another cutaneous disease. It is important for pathologists to recognize the broad range of dermatologic conditions of this population when they return to the United States.

1189 Increased $CD25^+/CD4^+$ Treg Cells in Lymphoid Tissue of HIV+ Patients Treated with HAART and STI

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Background: $CD25^+$ lymphoid cells are a subset of $CD4^+$ regulatory T cells (Treg) that plays a major role in immune regulation. Alteration of Treg cells during HIV infection is controversial in peripheral blood but has never been studied in lymphoid tissues.]Structured treatment interruption (STI) is an immunotherapy strategy used in HIV+ patients in early stages of the infection treated with antiretroviral therapy (HAART) that prevents or delays viral rebound in plasma. This study investigates the influence

of HAART and STI on the distribution of $CD25^+/CD4^+$ Treg cells in lymphoid tissue and its relationship with the tissue viral load.
Design: Twenty two tonsil biopsies of 9 HIV+ patients obtained before treatment (n=5), after 12 months of effective HAART (n=8) and after STI (n=9) were studied. All patients had baseline CD4 T-cell counts >500x106 cells/l and plasma viral load >5000 copies/ml. Lymphoid tissue viral load (LTVL) was determined in frozen samples by using the NucliSens HIV-1 RNA QT assay. Immunohistochemical studies were carried out using antiCD25 (Novocastra, Newcasle, UK, 4C9) and antiCD4 (Novocastra, 1F6) antibodies. Positive cells were counted in interfollicular areas and the mean value per HPF was calculated.
Results: Tonsil biopsies before treatment showed an absence of lymphoid follicles. After HAART and STI we observed an improvement of immunoarachitecture with recovery of follicular structures. Immunohistochemical analysis demonstrate an increase of $CD25^+$ Treg cell population in the interfollicular areas (p=0.01). The $CD4^+$ positive cells were also increased (p=0.061) (Table1). LTVL (p=0.015) was lower after HAART and STI and there was a significant correlation between $CD25^+$ Treg cells and LTVL (p=0.002).
Conclusions: The number of $CD25^+CD4^+$ Treg cells in lymphoid tissue of HIV-infected patients significantly improves after HAART and STI, and this recovery correlates with a decrease of the LTVL. These results suggest that $CD25^+$ Treg may play a role in the control of viral load and may be a useful index of immunological recovery on lymphoid tissue biopsies from HIV patients.

Table 1

	LTVL (mean copies/mg.)	CD25+ (cells per HPF)	CD4+ (cells per HPF)
1	7939509	1,4	181,4
2	70655,75	7,72	290,74
3	75095,5	12,82	366,54

LTVL: lymphoid tissue viral load; 1: biopsy at baseline; 2:biopsy after HAART; 3: biopsy after STI

1190 Mast Cells in HG Lesions of Patients Co-Infected with Human Papilloma Virus (HPV) and Human Immunodeficiency Virus (HIV)

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Background: HPV is the most common sexually-transmitted infection in the US. Persons co-infected with HIV have higher rates of HPV (often with multiple types); immunosuppression (IS) leads to persistent HPV and development of low grade (LG) or high grade (HG) intraepithelial lesions (SIL) and cancer (CA). Mast cells are predominantly distributed in the subepithelial tissue near blood vessels and nerves; their primary function is to store and release a number of biologically active mediators such as serine proteases. These compounds have been implicated in allergic reactions, angiogenesis, and tumor invasion. We reviewed a series of patients with HIV-HPV co-infection to determine if there is any correlation between disease manifestation and immune status, HPV type, mast cells and lymphocyte subpopulations.
Design: We identified 75 cases of HPV-related anogenital lesions in our HIV database (18 females: 8 IS; 27-48 yrs; avg 32.7; and 56 males: 27 IS; 19-66 yrs, avg 39.0). HPV results were available for 35 cases: 2/7 F had 2 HPV types; 16/28 M had multiple types. A subset (4 F: 4 IS; 25% 2 HPV types; 27-38 yrs, avg 33.5 and 11 M: 7 IS; 55% mult HPV types; 22-55 yrs, avg 38.5) was selected for further evaluation: 1) identify histologic changes associated with HPV types in individual lesions; 2) perform immunostaining for CD3, CD8, CD56, and mast cells (quantity, 0 to 3+, and distribution).
Results: All females had HGSIL of cervix and HPV 16/18. Ten males had anal lesions and 1 a penile lesion. Overall, HG lesions were present in 7/9 patients with IS compared to 3/10 with normal immunity; 7 had HPV 6/11 (5 LG, 1 HG, 1 a giant condyloma/CA), 11 had HPV 16/18 (2 LG, 7 HG,1 CA), 6 had HPV 31/33/51 (3 LG, 2 HG, 1 CA). Of the 7 patients with multiple HPV types, 5 had different lesions associated with the various HPV types. Mast cells were increased at the basement membrane (BM) only in flat LG or HG/CA (anus and penis, not cervix). Significant degranulation occured only in HG lesions and only at the BM. Exophytic lesions, including the giant condyloma had no mast cells near BM. Mast cell distribution varied by lesion type in the same patient. CD3+, CD8+ and CD56+ lymphocytes did not differ significantly by grade of lesion or type of HPV.
Conclusions: Factors associated with HG/CA lesions: immune suppression, HPV type 16/18 and/or 31/33/51 (8/9), and increased degranulating mast cells at BM.

1191 Vascular Endothelial Growth Factor, KDR, and Cycloxygenase-2 Expression in Reversal Reaction

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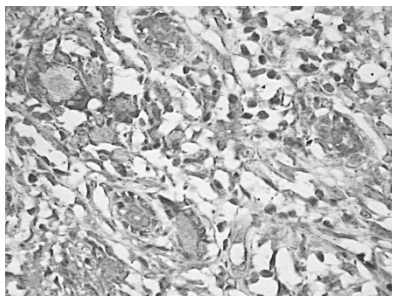
Background: Reversal reaction (RR) is a complication occurring in the borderline (BT-BL) area of the leprosy spectrum. It represents an acute episode, often amounting to a medical emergency, which alters the chronic, uneventful course of the disease and demands prompt treatment to prevent permanent nerve damage. The underlying immunological alteration is a delayed hypersensitivity response against *Mycobacterium leprae* antigens.
Design: Vascular endothelial growth factor (VEGF), its endothelial receptor, KDR, and cycloxygenase (COX)-2, an inducible enzyme synthesizing eicosanoids in inflammation, were demonstrated by immunohistochemistry and in situ hybridization in RR (n=7) in comparision with cases of non-reactive leprosy across the spectrum of the disease (n=7 BT, n=3 BL, and n=4 LL).
Results: In our experience, VEGF, KDR and COX-2 were expressed in cells of the mononuclear-macrophage lineage, including macrophages and epithelioid cells. Granuloma cells positivity was consistent across the spectrum of leprosy, from the vacuolated macrophages of lepromatous leprosy to the predominantly epithelioid cell infiltrate of tuberculoid leprosy. A distinctive feature of RR was VEGF, KDR and COX-2 positivity of the foreign-body type giant cells and of the tall-endothelium microvessels associated with edema.



Conclusions: These data indicate that the cascade VEGF ⇒ COX-2 is a major player in RR, which is characterized morphologically by the appearance of edema and foreign-body type giant cells. Experimental models support the view that VEGF and COX-2 expression are related events, with VEGF enhancing prostaglandin production via COX-2 stimulation and prostaglandin synthase expression. VEGF increase is associated with a parallel, dose-dependent increase in the expression of COX-II and membrane-associated prostaglandin synthase mRNA in cell culture. Furthermore, treatment with the COX-2 inhibitor NS-398 abolishes the VEGF-enhanced expression of prostaglandin synthase. Our findings support the view that COX-2 inhibitors should be investigated for RR therapy.

1192 Regional Prevalence of Sarcocystis Parasites in Retail Beef

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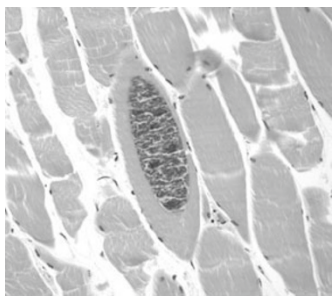
Background: Species of *Sarcocystis* are parasitic protists with 2-host life cycles. When an intermediate host ingests contaminated food or water, sporocysts invade striated muscle and form sarcocysts. Consumption of undercooked sarcocyst-containing meat leads to a gastrointestinal infection in the definitive host. Human may serve as the definitive, and rarely, the intermediate host for *Sarcocystis hominis*. Although *S. hominis* has been identified internationally, it has yet to be described in the United States. Bovine sarcocystosis due to *S. cruzi*, however, is widely prevalent and indistinguishable from *S. hominis* by light microscopy. The goal of this study was to determine the prevalence and identity of *Sarcocystis* species in retail beef using novel molecular amplification and sequencing methods.
Design: DNA extraction and ribosomal DNA amplification by polymerase chain reaction (PCR) was performed on 112 organic and conventional beef samples (112 animals) purchased at local and national grocery stores in Vermont, as well as on uninfected human liver specimens (negative controls). In 50 cases, adjacent tissue sections were examined for sarcocysts by routine light microscopy. DNA sequencing was performed on representative cases for species confirmation.
Results: Of the 112 beef specimens, 61 supported amplification of parasite rDNA by PCR. Sequencing 33 of these definitively identified all as *S. cruzi* and none as *S. hominis*. Of the 50 cases examined in parallel by each methodology, light microscopy and molecular amplification identified sarcocysts in 18 and 27 cases respectively. In 16 instances, infection was detected by neither assay. Six cases identified by light microscopy were not amplified by PCR, whereas 15 cases detected by PCR did not contain identifiable cysts on tissue section.
Conclusions: We confirm that *S. cruzi* is highly prevalent in retail beef, that zoonotic *S. hominis* only infrequently if ever occurs in domestic retail beef, and that PCR assays may increase detection sensitivity of bovine sarcocystis infections and facilitate their unambiguous identification.



1193 *Ataxia-Telangiectasia Mutated* Gene Product Activity Increases Resistance to *Aspergillus fumigatus* Gliotoxin Toxicity

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Background: *Aspergillus fumigatus*is is a common cause of invasive aspergillosis in immunocompromized individuals, causing significant morbidity and mortality. Gliotoxin, an *Aspergillus* metabolite, exerts toxic effects on immune cells, playing a significant role in aspergillosis. Gliotoxin is an oxidant which initiates dsDNA breaks, inducing apoptosis in immune cells. The gene product *ataxia-telangeictasia mutated* (ATM), absent in the rare disease *ataxia-telangiectasia* (A-T), is a kinase which plays a vital role in dsDNA break repair and oxidant resistance. We therefore tested the effects

of gliotoxin on A-T, normal, and A-T cells expressing recombinant ATM to examine the role of ATM in gliotoxin resistance.
Design: A-T, normal, and A-T cells expressing recombinant ATM were treated with gliotoxin and the effect on colony-forming efficiency, ATM kinase activity, and dsDNA break formation was examined. Additionally, the effect of quercetin and epigallocatechin gallate treatement (flavonoinds found in vegetables and green tea, respectively) was examined on cell colony-forming efficiency, dsDNA break formation, and ATM activation, both with and without later gliotoxin exposure.
Results: We found that; 1) ATM expression in A-T cells increased colony-forming efficiency following gliotoxin exposure, 2) gliotoxin activated ATM kinase activity, and 3) gliotoxin preferentially induced dsDNA breaks in A-T cells compare to normal cells and A-T cells expressing recombinant ATM. Additionally, pretreatment of normal cells with quercetin and epigallocatechin gallate increased cell gliotoxin resistance, and suppressed gliotoxin-induced dsDNA breaks. Similar results were obtained with A-T cells, though to a lesser extent. Interestingly, low flavonoids concentrations did not inhibit cellular colony-forming efficiency or cause dsDNA breaks, but did activate ATM in normal cells.
Conclusions: We demonstrate that ATM expression confers gliotoxin resistance. Additionally, two common dietary flavonoids confer gliotoxin resistance in normal and A-T cells and activate ATM in normal cells, demonstrating that these flavonoids increase cellular gliotoxin resistance via both ATM-dependent and -indendent mechanisms. Thus; 1) ATM expression confers gliotoxin resistance, 2) gliotoxin and two nontoxic flavonoids activate ATM, and 3) pharmacological activation of ATM via flavonoid intake may prove useful in the treatment of aspergillosis via lessening gliotoxin toxicity.

1194 Comparison of Immunofluorescence Antibody Testing and Two Enzyme Immunoassays in the Serologic Diagnosis of Malaria

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Background: Serologic testing in malaria may be utilized to aid in cases of low parasitemia, as a screening tool for blood banks, and in the retrospective diagnosis of malaria in a previously non-immune individual. It has traditionally been done by immunofluorescence antibody testing (IFA), but the use of commercially available enzyme immunoassays (EIA) has become more widespread.
Design: We compared IFA with two commercial EIA kits, the Cellabs Pan Malaria CELISA and the Newmarket Malaria EIA. Seventy-five samples from 74 patients with clinically suspected malaria were examined by both EIA kits. The samples were also examined by IFA (n=48) and/or Giemsa stained blood smear (n=48). Fifty healthy blood donor samples, 11 rheumatoid factor (RF) positive samples, and 11 anti-nuclear antibody (ANA) samples were also examined by the two EIA kits.
Results: Using a consensus result as the gold standard, the agreement, sensitivity, and specificity with 95% confidence intervals were, respectively: Cellabs EIA 93.2%, 95.5% (82.7 - 99.2%), and 92.2% (86.7 - 93.8%); Newmarket EIA 87.7%, 68.2% (54.1 - 74.6%), and 96.1% (90.0 - 98.8%); and IFA 89.1%, 86.4% (73.7 - 92.4%), and 91.7% (80.1 - 97.2%). Compared to positive Giemsa stained smears, the sensitivities were: Cellabs EIA 90.9% (10/11); Newmarket EIA 54.5% (6/11); and IFA 100% (11/11). ANA positive sera (n=11) and RF positive sera (n=11) showed no cross-reactivity with the Newmarket EIA, while the Cellabs EIA yielded positive results in one ANA positive and two RF positive sera. Among healthy blood donors (n=50), the Newmarket EIA showed 100% specificity (50/50) and the Cellabs EIA showed a specificity of 92% (46/50).
Conclusions: We conclude that while the Newmarket EIA was a generally more specific assay, it was insufficiently sensitive relative to the IFA and the Cellabs EIA for screening purposes for malaria antibodies. The Cellabs EIA demonstrated the best overall sensitivity and is a reasonable choice as a serodiagnostic tool for malaria. It may also be useful as an adjunct to Giemsa stained smear examination, to aid in cases of low parasitemia in previously non-immune individuals.

1195 Comparison of the Neutrophil Volume Distribution Width with Manual Band Count, C-Reactive Protein and Absolute Neutrophil Count for Predicting Acute Infection

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Background: The accurate diagnosis of acute infection is very important for proper patient management. Laboratory tests commonly ordered include complete blood count with differential, absolute neutrophil count (ANC), manual band counts, C-reactive protein (CRP) and blood culture. We have previously demonstrated that neutrophil volume distribution width (NDW) was significantly increased in bacteremic patients compared to controls. The NDW, which reflects neutrophil size variability, is quantitatively determined by Coulter hematology analyzer (Coulter LH750) with VCS technology during automated differentials. In this study, we compared the NDW to other laboratory parameters, such as manual band counts, CRP and ANC for predicting acute infection.
Design: We retrospectively analyzed data from 140 patients (M/F = 84/56; mean age = 50 years), who were subdivided, based on the medical history chart review, into three groups: group 1 (N = 27), no clinical evidence of infection; group 2 (N = 46), localized infection; group 3 (N = 67), severe or systemic infection. The data included the percent band count, CRP, ANC and the NDW generated by Coulter LH750. Statistical analyses were performed using ANOVA, Pearson correlation and ROC methods.
Results: The NDW ($p<0.001$), CRP ($p<0.05$), the band counts ($p<0.001$) and the ANC ($p<0.001$) were significantly increased in groups 2 and 3 compared to group 1. There were good correlations between the NDW and band counts as well as ANC ($r = 0.3$; $p<0.05$ and $r = 0.6$; $p<0.001$, respectively) in the severely infected patients. ROC analyses revealed that the NDW was the best with an area under the curve (AUC) of 0.84, which were greater than those of CRP (AUC = 0.65), the band counts (AUC = 0.74) and the ANC (AUC = 0.75).
Conclusions: Although the band counts and the ANC are also useful for diagnosing acute infection, the neutrophil volume distribution width (NDW) showed superior sensitivity and specificity. In addition, the NDW is a quantitative, more subjective and more accurate parameter generated by Coulter LH750 during automated differentials. We believe that the NDW may be used as an additional indicator for acute bacterial infection.

Kidney

1196 Routine Immunohistochemical Screening of All Renal Transplant Biopsies for Polyomavirus

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Background: Polyoma (BK) virus nephropathy (BKN) is a common and serious complication, occurring in 1% to 8% in renal allograft recipients, and often leads to severe allograft dysfunction and graft loss. The diagnosis of BKN relies on the identification of characteristic viral cytopathic effect in the renal tubular epithelium on light microscopic examination of a graft biopsy. Diagnostic confirmation can be achieved by immunohistochemistry for Simian virus (SV40) and electron microscopy. The incidence of BKN in renal allograft recipients that do not exhibit the diagnostic histological features, and can be diagnosed only by immunohistochemical or molecular techniques is not known.
Design: All non-protocol renal allograft biopsies over a 12 month period were stained for polyomavirus (SV40, clone PAb 280, Oncogene Research Products, Boston, MA) by immoperoxidase. In addition selected cases from a additional 10 month period were stained for polyomavirus. All light microscopic slides were carefully reviewed by two pathologists independently.
Results: Total number of 302 renal allograft biopsies was identified. Thirteen (4.30%) biopsies with the diagnosis of BKN, from 11 patients were identified, The patients age ranged between 27 and 72 years (mean 46.1 year), and there were 10 males (90.9%), and 1 female (9.09%). The time of allograft renal biopsies ranged from 3 to 53 months post transplantation (mean 18.4 months). On light microscopic examination, 8 (61.5%) biopsies from 7 (63.6%) patients showed classical histological features of polyomavirus cytopathic effect. Five (38.4%) biopsies from 5 (45.4%) patients exhibited a variable degree of reactive changes of the tubular epithelial cells, but no diagnostic viral cytopathic changes. All but one of these 5 patients showed clinical evidence of polyomavirus infection, including positive plasma polymerase chain reaction (PCR) test for BK virus DNA. All 13 renal allograft biopsies were positive for SV40 immunohistochemical stains.
Conclusions: Polyomavirus nephropathy is common complication in allograft renal transplant recipients. Routine screening by immunohistochemical staining for SV40 is recommended for every allograft renal transplant biopsy that performed for renal dysfunction, since it detects a significant number of polyomavirus infection, which is clinically relevant, but otherwise not detected by routine light microscopic examination.

1197 Immunohistochemical Staining for C4d on Formalin-Fixed Paraffin-Embedded Versus Frozen Sections in Renal Allograft Biopsies

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Background: Staining for C4d in renal allograft biopsies, in addition to histologic findings and anti-HLA serology, is considered to be a marker of humoral rejection. Most centers utilize a monoclonal antibody to C4d on frozen sections using either immunoflourescence or immunoperoxidase techniques. Staining paraffin sections can potentially be advantageous due to improved morphology, ease of interpretation, and reducing dependence on requiring frozen tissue. Although the specificity of the polyclonal C4d antibody on paraffin is thought to be high[1], its sensitivity for antibody-mediated rejection is unknown.
Design: We examined 48 non-protocol renal allograft biopsies that were diffusely or focally positive for C4d on frozen sections using a monoclonal antibody (Quidel, Santa Cruz) by immunoperoxidase from two centers. For comparative purposes, an additional 52 consecutive C4d negative biopsies were also studied. Formalin-fixed paraffin sections were stained by immunoperoxidase using a polyclonal antibody against C4d (BIOMEDICA, Salem, NH). Clinical and histological factors were used to help determine whether antibody mediated rejection occurred.
Results: Forty-eight of 100 cases were found to be C4d positive on frozen section using the monoclonal antibody and fifty-two cases were negative. Forty-two of the 48 cases subsequently showed positive staining using the polyclonal antibody on paraffin. Twenty-four of 42 showed strong positive staining (2+ or 3+), 6 showed weak staining (1+) and 12 showed equivocal staining (focal 1+). Of the remaining 6 of 48 cases, 3 showed no paraffin staining for C4d, and 3 were difficult to interpret due to scant tissue. All cases positive on paraffin sections also showed glomerular staining, which often was comparable in intensity to the peritubular capillary staining. Of the 3 cases negative on paraffin sections for C4d but positive on frozen section, all showed either clinical or histologic evidence of antibody mediated rejection. All of the 52 cases that were negative for C4d staining on frozen section were negative on paraffin sections.
Conclusions: Paraffin staining for C4d is not as sensitive for antibody mediated rejection as using the monoclonal antibody against C4d on frozen sections.