1294 Expression of Intracellular Domain of Notch1 (ICN1), Lef-1, and Phosphorylated STAT3 (Pstat3) in Angioimmunoblastic T-Cell Lymphoma

XM Zhang, X Li, L Chiriboga, P Chandra, P Lee, S Ibrahim. New York University School of Medicine, New York, NY.

Background: Pathologically, angioimmunoblastic T-cell lymphoma (AITL) is characterized by a pleomorphic population of small to medium sized predominantly CD4+ T-lymphocytes with a smaller population of larger cells with clear cytoplasm concentrated around expanded follicular dendritic cell (FDC) meshworks and extensive amount of high endothelial venules. Clinically, AITL is characterized by rash, hepatosplenomegaly, hypergammaglobulinemia, and/or lymphadenopathy. Recent studies including ours, suggest that the origin of AITL is from germinal center T-helper cells (GC-Th). Gene expression analysis reveals that GC-Th cells express high level of Notch1. Oncogenic Notch mutations, which cause aberrant over-expression of ICN1, and its target genes including Lef-1 and pSTAT3 have been shown to play imporatnt roles in the transformation and survival of lymphoma cells. This study utilized a panel of antibodies including ICN1, Lef-1, and pSTAT3 to investigate the presence of oncogenic notch mutation and elucidate the downstream signal activation patterns involved in AITL.

Design: Using 4mm punches on archived paraffin blocks, a tissue microarray was constructed comprising 38 cases including 14 cases of AITL, 11 cases of peripheral T-cell lymphoma, unspecified (PTCL-u) and 13 cases of reactive lymphadenopathy (RL). Immunostains for ICN1, pSTAT3, and Lef-1 were performed on the tissue microarray.

Results: PSTAT3 is positive in 50% of AITL, negative in PTCL-u, and positive in 8% of RL. In the positive RL cases, pSTAT3 is expressed only on endothelial cells. Lef-1 is positive in 90% of AITL, 70% of PTCL-u, and 50% of RL. In the reactive lymph nodes, Lef-1 expression is restricted to the interfollicular areas with occasional positivity seen in germinal centers. Interestingly, some of Lef-1 positive PTCL-u cases are histologically similar to AITL, which have been classified in the literature as "AITL-like PTCL". ICN1 stain shows mainly cytoplasmic expression with occasional nuclear stain, seen in 90% of AITL, 10% of PTCL-u, and 8% of RL. One AITL case is strongly positive for pSTAT3, Lef-1, and ICN1. This particular case was shown in our previous study to be strongly positive for CD4, CD10, Bcl-6, and CXCL13.

Conclusions: Our findings suggest that Notch1 signaling pathway is operative in the pathogenesis of some AILT cases if not all. Notch1 may be used as a marker to distinguish AITL from PTCL-u and RL. PSTAT3 has good diagnostic utility in the distinction of AILT, PTCL-u, and RL.

1295 Loss of Chromosome 1p21 Band Is Associated with Disease Progression and Poor Survival in Multiple Myeloma Independent of High-Risk Genetic Factors

J Zhao, C Qi, A Jiang, W Xu, T Young, D Reece, H Chang. Dept of Laboratory Medicine & Pathobiology, University of Toronto, Toronto, Canada; University Health Network, Toronto, Canada.

Background: Amplifications involving chromosome 1q and deletions involving 1p are frequent events in multiple myeloma (MM). The pathogenesis and clinical significance of these anomalies are largely unknown. As karyotyping and SNP based mapping analysis identify a minimal common deletion region involving the 1p21 locus, we investigated the prevalence and prognostic significance of del(1p21) in a large cohort of MM patients.

Design: Bone marrow aspirates were obtained from 203 MM patients undergoing autologous stem cell transplant at our institution. Fluorescence in situ hybridization combined with cytoplasmic light chain detection (cIg-FISH) was used to evaluate clonal plasma cells for 1p21 deletion as well as other myeloma-associated chromosomal abnormalities. In addition, 1p21 status was evaluated in 16 patients with of monoclonal gammopathy of undetermined significance (MGUS) and 32 patients with plasma cell leukemia (PCL).

Results: CIg-FISH detected hemizygous 1p21 deletions in 18% of the MM, 38% of PCL but none of the MGUS cases. In MM, the median percentage of clonal plasma cells harboring del(1p21) was 55% (range 20-95%). The presence of 1p21 deletions was strongly correlated with 1q21amplification (p=0.005), t(4;14) (p= 0.03), and del(p53) (p=0.04), but not with del(13q) or t(11;14). There was no association between del (1p21) and other biological factors including age, gender, Hb, albumin, C-reactive protein, beta-2 microglobulin level, isotype or bone marrow plasmacytosis. Patients with 1p21 deletions had significantly shorter progression-free (median 13.9 vs. 25.4 months, p<0.001) and overall survivals (median 33.9 months vs. not reached, p=0.001) than those without such deletions. On multivariate analysis, del(1p21) was an independent risk factor for progression free (p= 0.001) and overall survivals (p=0.040) after adjusting for other genetic risk factors including del(13q), del(p53), t(4;14) and 1q21 amplification.

Conclusions: Our results indicate that del(1p21) is a novel genetic risk factor associated with disease progression and adverse outcome, thus warrant inclusion of this genetic aberration in the risk-stratification of MM. Further studies are required to identify candidate tumor suppressor gene(s) at the1p21 locus and explore their role in the molecular pathogenesis of MM.

Infections

1296 Histiocytic Necrotizing Lymphadenitis (Kikuchi-Fujimoto Disease): Report of 44 Cases from Saudi Arabia

SS Amr, SS Sheikh. Dhahran Health Center, Dhahran, Eastern Province, Saudi Arabia.

Background: Histiocytic necrotizing lymphadenitis also known as Kikuchi-Fujimoto's disease (KFD) was first described in Japan in 1972. Since then KFD had been reported from many countries world wide. In the Arab World, cases had been reported from Saudi Arabia, Jordan, Tunisia, Lebanon, UAE, and Oman.

Design: We report 44 cases of KFD diagnosed at our hospital during 20 year period (1987-2007). We reviewd the histological sections, demographic data and clinical and laboratory findings of our cases. We submitted 14 cases for nested PCR studies for EBV (EBNA1) as a part of collaborative study with centers in UK and USA.

Results: There were 27 females and 17 males (M:F ratio 1:1.6), their ages ranging between 4 and 42 years. There were 15 children under the age of 18. No specific diseases were associated with our cases except for three patients: Two girls, 6 and 8 years old, subsequently developed systemic lupus erythematosus and the third patient, a 39 year old woman, was diagnosed with mixed connective tissue disease and Hashimoto's thyroiditis. Four patients had familial occurrence of the disease. Two sisters were diagnosed 10 years apart. Both were non-twin sisters who had identical HLA phenotype (A31, B35, B49, C4, C6, DR15, DR13, DRW51, DRW52, DQ6). A sister and brother had KFD within one year. In addition, we had two patients who had recurrent disease. One patient recurred 5 years later whereas the second one had two recurrences 4 and 8 years after the initial diagnosis. We observed seasonal variations in the occurrence of the disease with more than two third of our cases diagnosed during the fall and winter seasons. 14 cases were tested for EBV using nested PCR for EBNA1. Four cases tested positive for EBV (EBNA1).

Conclusions: This study of 44 cases of KFD illustrates the spectrum of this uncommon disease in Saudi Arabia, with certain peculiar findings including association with pediatric SLE, mixed connective tissue diseas and a unique familial occurrence with identical HLA phenotype. We are investigating the etiology of KFD with other workers, utilizing PCR for various viruses. Further studies are needed to elucidate the etiology of this disease.

1297 Clinicopathologic Analysis of Patients with BK Viruria and Rejection-Like Graft Dysfunction

I Batal, ZM Franco, R Shapiro, A Basu, H Tan, A Vats, A Zeevi, C Morgan, P Randhawa. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: BK virus (BKV) nephropathy is usually treated with decreased immunosuppression (IS). In viruric patients with no tubulitis or viral inclusions, and negative in-situ hybridization for viral DNA, immunosuppression is frequently increased but therapeutic response has not been systematically studied.

Design: We evaluated 25 viruric patients with 40 episodes of graft dysfunction treated with increased IS. Biopsies showed changes interpreted as acute cellular rejection Banff type 1A (20), 1B (19), or 2A (1). We defined 2 analysis groups (Gp): low viral load (VL) (Gp A <1.0E5 copies/ml, n=28) and high viral loads (Gp B >1.0E5 copies/ml, n=12). Creatinine (Cr) response was assessed semi-quantitatively [-1 to+2] corresponding to worse, stable, partial response and complete response respectively. Follow-up viral loads (VL) were assessed using a semi-quantitative scoring system [-1 to +1] corresponding to increase of VL >3 fold, no change, or decrease>3 fold. Similarly, follow-up biopsies were scored from [-1 to+1] corresponding to worsening, no change, or improved findings.

Results: Compared to Gp A, Gp B showed a trend toward worse Cr response (-0.08 +/- 1.08 B versus 0.57 +/- 1.26 A; p=0.17) and worse VL response (-0.88 +/- 0.35 B versus 0.22 +/- 0.8 A; p=0.07). Follow up biopsies obtained 36 +/- 28 days later showed similar incomplete response to anti-rejection therapy [Gp A: 0.27 +/- 0.7, Gp B: 0.25 +/- 0.89]. None of the patients within Gp A became viremic following anti-rejection treatment while 6/11 (55%) patients became viremic in Gp B. Immune cell function values within 14 days of index biopsies were available for 12 episodes [(Gp A (n=7), Gp B (n=5)] with a mean value of 200 +/- 140 ng/ml ATP [much < previously described for rejection episodes without viruria (median 462 ng/ml ATP)]. These values tended to be lower in Gp B (129 +/- 99 ng/ml ATP) than Gp A (243 +/- 149 ng/ml ATP) (p=0.16). Peritubular capillary C4d deposits were found in 11/29 (38%) of Gp A vs 3/10 (30%) of Gp B. Biopsies in the two groups did not differ with respect to grades of inflammation, tubulitis, or interstitial fibrosis.

Conclusions: Patients with BK viruria and tubulitis show incomplete response to increased IS, suggesting that the observed pathology is, in part, initiated by viral rather than allogeneic antigens. Higher urine BKV load seems to be associated with lower creatinine response, and greater subsequent incidence of viremia.

1298 Partners in Pathology: A Collaborative Model To Bring Pathology to Resource Poor Settings

JW Carlson, E Lyon, D Walton, W Foo, P Farmer, V Nosé. Brigham and Women's Hospital, Boston, MA.

Background: Pathology provides a critical bridge between patients, their physicians and the therapeutic and surgical interventions that can be provided to them. However, because pathologists often work as professional consultants, they are often not considered to have a role in alleviating health disparities for underserved patients. Partners in Health is a comprehensive, community based health care organization with clinics in seven countries. The disease burden in these settings is primarily infectious, however, pathology plays a key role in select situations. Partners in Health clinics have the ability to obtain surgical biopsies through a collaborative effort with our Department, and provide treatment and follow up, even in some of the poorest parts of the world.

Design: To explore the utility of pathology in a setting where the disease burden is primarily infectious and to demonstrate the ease of providing pathology services in resource poor settings, through collaboration with clinicians working on-site.
Results: Thirty consecutive cases from Haiti and Rwanda from patients with a median age of 30 years were examined. Diagnoses included 18 malignancies, 9 infectious or inflammatory cases, and other rare entities. Among the infectious cases were two cases of tuberculosis, a case of chromoblastomycosis, a case of actinomyces, and several cases with necrotizing granulomas where no organisms were identified. Additionally, two neoplasms were demonstrated to be EBV related.
Conclusions: The disease burden in resource poor settings is primarily infectious. In this cohort, although the majority of lesions that were biopsied were neoplastic in nature, some rare infectious entities were diagnosed and treated accordingly thanks to this initiative. This collaboration between an academic pathology department and physicians working together was simple, and brought with it a host of benefits.

1299 Fungal Mimics in Granulomatous Lymphadenitis: Prevalence of Hamazaki-Wesenberg Bodies and Morphologic Features That Aid in Their Differential Diagnosis

KS Cole, L Fajardo, J Hecht, MR Mariappan. Harvard Medical School and Beth Israel Deaconess Medical Center, Boston, MA; Stanford University Medical Center & VA Palo Alto, Palo Alto, CA.
Background: Hamazaki-Wesenberg (HW) bodies are spindle shaped, lipid rich, non-infectious structures that were reported in the early 1970s in reactive lymph nodes, specifically granulomatous lymphadenitis associated with sarcoidosis. They resemble fungal elements morphologically, are argyrophilic and can potentially be mistaken for fungal infections. The HW bodies are of unknown origin, but thought to be derivatives of lipofuscin. We studied and report the current prevalence of HW bodies in granulomatous reactive nodes and highlight the distinguishing features from fungal elements.
Design: To study the incidence of HW bodies, we retrospectively collected lymph nodes with sarcoidosis from our recent archives (4 years; n=34). Morphological examination on routine Hematoxylin and Eosin/Grocott Methenamine Silver (GMS) were performed. We also compared the morphology of HW bodies with that of histoplasma spp.
Results: The demographics were as follows: mean age: 50.8 years (range 34-83); 20 females and 14 males. Most (30/34) lymph nodes biopsied were from the mediastinum (para- and pre-tracheal, superior mediastinal and sub-carinal). The 4 non-mediastinal locations were supra-clavicular, anterior cervical, inguinal and retro-peritoneal. HW bodies were identified in 15/34 lymph nodes (prevalence: 44%). The HW bodies were GMS positive and ranged from 2-12 µm. They were mostly in sub-capsular sinuses, yellow-brown and refractile. Morphological differences between histoplasma and HW bodies: Histoplasma spp. and other yeast are usually in tight clusters, mostly intracellular, oval or rounded, show occasional budding and associated with inflammation and necrosis. HW bodies are single or in loose clusters, present intra- and extra-cellularly and are round to spindle shaped bodies, without inflammation or necrosis. GMS stains highlight an important difference: the fungal elements have a dark cell membrane with central palor/clearing. The HW bodies, derived from complex lipids without cellular organelles, are uniformly dark staining.
Conclusions: Hamazaki-Wesenberg bodies, described in the 1970s, are still commonly seen in reactive nodes. In our series, the incidence of HW bodies in patients with granulomatous lymphadenitis is ~44%. HW bodies as a mimic should be borne in mind when fungal-like elements are noted in reactive lymph nodes.

1300 Histopathologic and Immunohistochemical Characteristics of Pneumonias in Relation to the Etiologic Agent

J Guarner, WJ Shieh, CD Paddock, SR Zaki. Emory University School of Medicine, Atlanta, GA; Centers for Disease Control and Prevention, Atlanta, GA.
Background: The histopathology of infectious pneumonias encompasses several features that can be characteristic depending on the etiologic agent. The objective of this study is to compare the histopathology of pneumonias based on organism-specific immunohistochemically (IHC) assays.
Design: We describe the histopathologic features of formalin-fixed postmortem lung samples that showed positive IHC staining for the most frequent bacteria and viruses.
Results: We identified 171 IHC-positive pneumonias from 1998 to 2006. Intraalveolar collections of neutrophils associated with positive staining for *Streptococcus pneumoniae* were observed in 44 cases. Influenza virus staining was detected in 46 cases, primarily in broncho-epithelial cells of central airways, and usually associated with various degrees of peribronchial lymphohistiocytic inflammation. In 10 cases with influenza virus infection, another infectious agent was observed and the pathology changed depending on the second microorganism present. Intense necrotizing tracheobronchitis usually accompanied with accumulation of neutrophils in the alveoli was observed in 24 *Staphylococcus aureus* cases. Abscesses of various sizes characterized the 23 *Streptococcus pyogene*s cases. Staining for *Bordetella pertussis* was observed in 14 children who showed intraalveolar edema, hemorrhage, and various amounts of inflammation. *B.pertussis* usually coated the airways or were present inside inflammatory cells. Staining for *Legionella pneumophila* was present in 12 cases which showed macrophages as the primary intraalveolar inflammatory infiltrate. Staining for respiratory syncytial virus, herpes simplex, adenovirus, and *Haemophilus influenzae* was observed in 6, 5, 4, and 3 cases respectively. Viral inclusions were observed in cases of herpes simplex and adenovirus infections, and multinucleated giant cells with or without viral inclusions were observed in herpes and respiratory syncytial virus infections.
Conclusions: Pathogen-specific IHC of postmortem lung specimens is a versatile and specific technique for the diagnosis of pneumonias that can be used when cultures are not conclusive. The etiologic agent of the pneumonia will determine the histopathologic features observed in the lung.

1301 Diagnosis of Pediatric Malaria in the 21st Century

J Guarner, J Gutman. Emory University School of Medicine, Atlanta, GA.
Background: In recent years, immigration has increased to the United States and travel of these families to their home countries has increased reaching 40% of international travel in 2002. These travelers are at risk of acquiring infectious diseases prevalent in their home countries thus US clinicians and laboratories need to be familiar with these diseases. Although malaria is the most frequent serious infection to travelers, it is misdiagnosed as often as 60% of the time on initial presentation, especially in children.
Design: We performed a retrospective review of malaria confirmed cases from 2 Atlanta pediatric hospitals.
Results: Tests for malaria (thin and thick smears) were performed in 331 patients and malaria parasites were present in 44 (13.3%). Diagnosis of malaria was primarily based on presence of ring forms since gametocytes were rarely identified. *Plasmodium falciparum* was diagnosed in 31 (70%) cases, *P. vivax* in 4, *P. ovale* in 3, *P. malariae* in 1, and *Plasmodium sp*. in 5. Parasitemia below 1% was found in 23 (52%) cases, between 1 and 5% in 14 (32%), between 5 and 10% in 2, and above 10% in 3. The child with the highest parasitemia (28%) was febrile and had headache and abdominal pain but no signs of cerebral malaria. The mean hemoglobin concentration was 10.4 g/dl (range of 5.4 to 13.8). C-reactive protein was available in 29 patients and showed a mean of 7.7 µm/ml (range of 0.5 to 25.4). The mean age of the patients was 8.3 years (range 1 to 16) with 28 males. Six patients were refugees or recent immigrants, 2 patients were foreigners visiting the US, and 36 (82%) were children that had traveled to visit family and friends. Nigeria was the country most frequently visited (19 cases), followed by Cameroon (6). There were only 2 patients that had traveled to Central America or the Caribbean. In 3 patients travel had occurred 8 to 12 months before they were diagnosed with malaria. Thirty four (72%) patients presented to the hospital between May and August. Treatment was primarily with quinine and clindamycin or doxycycline. The only patient requiring transfusion therapy had the lowest hemoglobin (5.4 g/dl).
Conclusions: Malaria diagnosis can be difficult in children because parasitemia is usually below 1%. Since gametocytes are rarely observed, speciation is usually based on presence of ring forms. Even though immigration from Latin America is higher than that from Africa, most of the pediatric malaria cases were in patients that had visited Africa during the summer (May through August).

1302 Solitary Necrotic Nodule of the Liver: Does *Strongyloides* Infection Play a Part in the Aetiology?

MJ Hale, AC Paterson, DA Turton, AD Danckwerts, JM Rigby. School of Pathology, Faculty of Health Sciences, University of the Witwatersrand and National Health Laboratory Service, Johannesburg, South Africa.
Background: Solitary necrotic nodule of the liver was first described by Shepherd and Lee in 1983. It is a rare entity that is often identified as an incidental finding, either by the use of imaging techniques during the clinical investigation of suspected liver disease or at autopsy. A number of reports have appeared in the literature and currently only about 30 have been described. Various postulates have been proposed as to the aetiology, but to date a specific cause has yet to be identified.
Design: Three cases of solitary necrotic nodule are presented. A 41yr old white female who presented with sclerosing cholangitis. As part of the work-up for liver transplant two nodules were identified on two occasions and diagnosed on biopsy. The explanted liver showed multiple tan to white, well demarcated nodules measuring up to 3mm in diameter. A 32 yr old female patient presented with a single liver nodule. No further information was available. A 42 yr old male patient who underwent a splenectomy following a road traffic accident. At laparotomy firm lesions were noted on the liver.
Results: The histopathological findings of all three cases were similar, characterised by a well demarcated central area of necrosis surrounded by a thickened cuff of fibro-elastic tissue showing granulomatous inflammation which in two cases was associated with an infiltrate of eosinophils. The second case showed evidence of infection by necrotic nematodes with morphological appearances suggestive of *Strongyloides* infection.
Conclusions: These cases suggest that solitary necrotic nodule may have a parasitic aetiology and that *Strongyloides* infection should be considered in the aetiopathogenesis. Furthermore the term "solitary" may be a misnomer and that multiple lesions with the same histopathological features may be present in the same patient.

1303 Use of Neutrophil and Lymphocyte VCS Indices Aids in the Diagnosis of Pediatric Bacterial Infection

SC Koenig, D Pongvongkeo, R Magari, K Quillen. Boston Medical Center, Boston, MA; Coulter Diagnostics, Miami, FL.
Background: In previous work (USCAP 2005), our department showed that the size of neutrophils (mean neutrophil volume, MNV) increases during acute systemic bacterial infection, and that the sensitivity of MNV is superior to the WBC and the neutrophil percentage (%Neu). Here we focus on bacterial infections in ambulatory pediatrics, and expand our VCS indices to include the NDW (neutrophil distribution width) as well as lymphocyte parameters (mean lymphocyte volume, MLV, & lymphocyte distribution width, LDW).
Design: The study population includes 147 patients age <19 presenting to the emergency room with fever ≥100.5 and had an infectious work up including at least one of the following: blood culture, chest x-ray, CSF, rapid strep test, throat culture, urinalysis/culture, clinical exam for otitis media. Patients were identified by reviewing all CBC/differential from the pediatric ER during a 3-month period. Patients who were pregnant or had received antibiotics in the past 7 days were excluded. The work up was considered positive if any of the tests indicated a bacterial infection. VCS data collected included WBC, Absolute Neutrophil Count (Abs Neut), % Neu, MNV, NDW, Absolute Lymphocyte Count, % Lymph, MLV & LDW. The sensitivity, specificity, AUC & p values were calculated.

Results: 70 patients had a positive work-up for bacterial infection; of these,19 had bacteremia. The 77 patients who had a negative work-up were assumed to have a viral infection (or no infection). There were significant increases in WBC, Abs Neut and NDW in those with a bacterial infection. NDW showed the best diagnostic ability but the AUC was relatively low. A logistic function used to combine NDW with LDW showed that the AUC for f (NDW, LDW) was better than NDW alone. The results for localized infections were similar to the results for all infections. The small number of bacteremic patients (19) did not allow for meaningful analysis.
Conclusions: The NDW, in addition to WBC & %Neu, may be used to diagnose bacterial infections in ambulatory pediatrics. Combining NDW with LDW enhances diagnostic ability. These morphologic indices are obtained automatically, at no extra cost, as part of a CBC/differential; their utility needs to be explored further.

VCS indices for all Infections

	WBC	%Neu	NDW	LDW
Mean (neg)	12.42	50.85	23.1	18.3
Mean (pos)	15.78	58.85	24.9	17.2
p value	0.002	0.014	0.007	0.007
AUC	0.634	0.603	0.672	0.618

1304 HPV DNA Testing in a Population with High Prevalence of Cervical Squamous Carcinoma: 5-Year Experience in Urban Peru

EML Li Ning, M Bazan, J Arias-Stella, Jr.. Instituto de Patología y Biología Molecular, Lima, Peru.

Background: Cancer of the cervix uteri is the second most common malignant neoplasia among women worldwide, with over 80% of deaths attributable to this cancer occurring in developing countries. Because the majority of cervical carcinomas are caused by human papillomavirus (HPV), high sensitivity techniques to detect the presence of this virus have been developed in the past decades. However, their high cost has limited the evaluation of their potential benefits in underprivileged areas. Peru has one of the highest prevalence rates of invasive squamous carcinoma in the world. Deficiencies on screening strategies along with a poor understanding of the epidemiology of HPV infection account for its high occurrence. For the last 6 years, our laboratory has provided HPV DNA testing as adjunct to cytology in urban areas across the country. We hereby present part of our experience on HPV DNA testing on cervical samples with abnormal cytology referred to our institute in a period of over 5 years.
Design: Hybrid Capture™2 (Digene, Inc., Gaithersburg, MD) assay was performed on cervical samples collected and referred to our laboratories from different cities in Peru. Samples were transported and processed following the manufacturer's recommendations from April 2001 to August 2006. Probe B for high-risk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 y 68) or probe A for low-risk HPV (6, 11, 42, 43, 44) were used. Patients' age ranged from 17 to 87 years (average 36.15 years).
Results: High-risk (HR) HPV was evaluated in 2208 cervical samples referred because of atypical pap smears (ASCUS). The overall presence of HR HPV was 33.6% (n = 741), that was distributed as follow: 44,4% among women aged 17 to 29; 34,04% among those aged 30 to 39; 25.06% among those aged 40 to 49; 20.7% among those aged 50 to 59 and 29.5% among those aged 60 to 87. Additionally, low-risk (LR) HPV was also evaluated on 388 of the 2208 samples. In 64 cases (16.5%) the presence of LR HPV was detected, of which 18 (4.6%) presented no concurrent HR HPV.
Conclusions: HR HPV is common among Peruvian females with ASCUS in pap smears, while LR HPV infection was found in only a minority of cases. HR HPV was more frequent in women aged 17 to 39 years. Interestingly, the prevalence of HR HPV on abnormal pap smears was very similar to what has been reported in the USA. Hybrid capture is a helpful tool to clarify cases of dubious cytology and can be used by reference centres for quality control of pap smears interpretation in low-resource settings.

1305 ThinPrep® and Surepath® Liquid-Based Media Validation Testing with GC/Chlamydia BD ProbeTec™ ET System

M Maamar, PL Cernoch, DJ Kim. The Methodist Hospital, Houston, TX.

Background: *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (GC) are the most common sexually transmitted bacteria in the United States. They are primary pathogens responsible for pelvic inflammatory disease and its sequelae including infertility. Moreover, approximately 70-80% of acute infections among women are asymptomatic. In accordance to universally accepted recommendations by the U.S. Preventive Services Task Force (USPSTF), sexually active adolescent and young women are routinely screened for cervical cancer with ThinPrep® (Cytyc Corporation) or Surepath® (Tripath Imaging) liquid-based pap tests. In both cases, a significant amount of the transport media remains after processing. This validation study evaluates the compatibility of these transport media with strand displacement amplification (SDA) of GC and Chlamydia DNA via the BD ProbeTec™ ET System.
Design: Patients' BD ProbeTec™ ET endocervial swabs were processed for routine patient work. Once processed, using a reconstruction format, each swab was swished and expressed into a ThinPrep® (PreservCyt®) or Surepath® (Preservative Fluid) transport media bottle. Then, a sample of the media was pipetted into an empty BD ProbeTec™ ET sample tube. The tube was centrifuged at 2000 x g for 30 minutes and the supernatant was decanted. The pellet was resuspended in BD ProbeTec™ ET sample diluent and vortexed prior to lysing. The BD ProbeTec™ ET assay protocol for urine specimens was then followed.
Results: Initially, 31/32 (97 %) and 19/19 (100%) of the GC positive specimens remained positive in PreservCyt® and Surepath Preservative Fluid inoculated solutions respectively. There was one low positive PreservCyt® specimen. Subsequent retesting yielded a positive result. Conversely, 37/37 (100%) and 22/23 (96%) of the Chlamydia positive specimens remained positive in PreservCyt® and Surepath Preservative Fluid inoculated solutions respectively. There was one low positive Surepath Preservative Fluid specimen. Subsequent retesting yielded a negative result. All negative PreservCyt® and Surepath Preservative Fluid specimens (75 each) remained negative.
Conclusions: With adequate prior processing, the study data reveals that the compatibility of ThinPrep® (PreservCyt®) and Surepath® (Preservative Fluid) transport media with the BD ProbeTec™ ET system is highly sensitive and specific. Nevertheless, the processing appears operator technique dependent. Careful removal of the liquid based preservative is critical since both proprietary buffered solutions contain methanol which is inhibitory to SDA techniques.

1306 HPV Testing – The Next Generation

P Manna, S Lex, D Stark, S Kerley, S Brachtesende, V Hunter. Physicians Reference Laboratory, Overland Park, KS; St Joseph Medical Center, Kansas City, MO.

Background: Several reports suggest that type-specific detection of all high risk HPV will help clinicians assessing the risk of their patients for developing cervical cancer. We have developed and validated the COMPLeTe Care (comprehensive oncogenic molecular papillomavirus laboratory testing, patent pending) HPV test and tested in our patient population. This test simultaneously detects and type-specifically identifies of all 15 oncogenic HPV types directly from Pap and tissue specimens.
Design: COMPLeTe Care HPV, a multiplex real time PCR test was first evaluated to determine its analytical performance characteristics such as sensitivity, specificity, reproducibility followed by side-by-side comparison with HC2 (Digene Corp) on 229 specimens. Furthermore, it was also validated against cytology and biopsy results. The test was then utilized on over 500 patients.
Results: COMPLeTe Care HPV test was determined to be highly sensitive (detects <10 viral copies), specific, reproducible, rapid, and clinically meaningful. It showed 100% agreement with CIN2/3 biopsy results. Excluding HPV types 73 and 82 (as they are not detected by HC2), parallel comparison with HC2 showed 92.4% positive agreement and 92.3% negative agreement, respectively, between the two; however, repeat testing and sequencing of all discrepant specimens favored COMPLeTe Care HPV. Although HPV 16 was most prevalent, however, HPV 18 was detected to be the sixth, not the second most prevalent oncogenic HPV type in our patient population (Table 1). Interestingly, 36% of positive patients had co-infections, of which 64% had double, 26% had triple, and 8% had quadruple HPV infections.

Table 1: Prevalence and distribution of high risk oncogenic HPV types simultaneously detected and typed by COMPLeTe Care HPV test

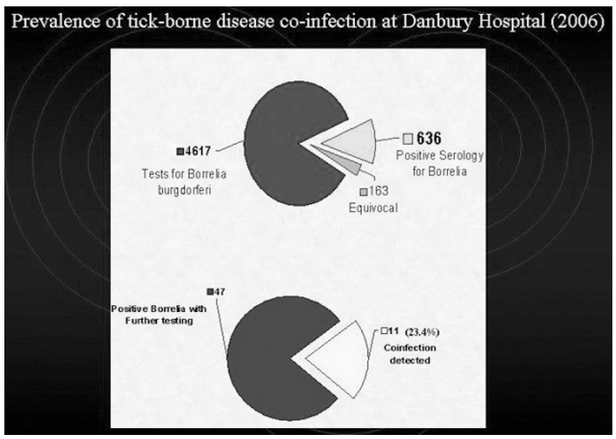
HPV Types	16	18	31	33	35	39	45	51	52	56	58	59	68	73	82
Single Infection	71	14	14	4	7	12	7	26	10	9	4	15	5	12	7
Co-Infections	59	18	24	7	9	27	10	31	31	13	17	23	6	14	10
Total	130	32	38	11	16	39	17	57	41	22	21	38	11	26	17
% of Positive	38%	9%	11%	3%	5%	12%	5%	17%	12%	6%	6%	11%	3%	8%	5%

Conclusions: Modern diagnostic possibilities such as real time PCR that identify the cause of the disease prior to the symptoms appearing can help to set a new standard in early type-specific detection of all high risk HPV types and will improve patient management and critically reduce morbidity, mortality, and costs.

1307 Prevalence of Tick-Borne Disease Co-Infection at Danbury Hospital a Seven Month Retrospective Study (April-October 2006)

JA Merayo-Rodriguez, LW Edwards. Danbury Hospital, Danbury, CT.

Background: Infections by tick borne vectors increase in incidence during the summer months causing significant disease in humans. The most common causative agents in the service area for the Danbury Hospital Laboratory are Borrelia burgdorferi (Lyme Disease), Anaplasma phagocytophilum (Acute human granulocytic ehrlichiosis) and Babesia microti (Babesiosis). It is important to differentiate between the various tick-borne diseases and arrive to an accurate diagnosis followed by appropriate antibiotic therapy, particularly in those patients who fail to respond to traditional treatments for Lyme disease. Reports have demonstrated that individuals infected with Borrelia burgdorferi have an approximately 9.4% chance of being coinfected by one or both Anaplasma or Babesia organisms.
Design: We retrospectively reviewed Danbury Hospital Laboratory Information System database (CERNER) for a seven month period (April to October, 2006) to identify patients whom underwent immunoserologic testing for Borrelia burgdorferi and correlated the positive results with testing for the other agents including smear preparation and molecular studies.
Results: A total of 4617 patients were tested for Borrelia, 292 for Anaplasma and 83 for Babesia by immunoserology. Six hundred and thirty six (636) cases were positive for Lyme serology (13.7 %) and 163 (3.5 %) cases were equivocal. Forty seven (47) of these 636 (7.3 %) had additional testing for one or both of the other two organisms. Three (3) patients showed evidence of coinfection with Borrelia burgdorferi and Anaplasma phagocytophilum by serologic testing. Review of smear preparation and molecular studies provided evidence of coinfection, 3 and 1 respectively, for a total of 4 out of 47 tested cases. Four patients' results revealed evidence of coinfection with Borrelia burgdorferi and Babesia microti on serology. Therefore, the coinfection rate observed at the Danbury Hospital laboratory is 23.4 % (11 out of 47 tested).
Conclusions: Comparison of our present results with the ones that we previously reviewed in 2005 suggests that Laboratory testing for all three organisms in our population has a rate of coinfection about twice the available published data.



1308 *In Situ* Hybridization for Aspergillus 18S Ribosomal RNA (rRNA) Sequences Using a Locked Nucleic Acid (LNA) Probe

KT Montone. University of Pennsylvania Medical Center, Philadelphia, PA.

Background: Locked nucleic acids (LNA) are modified RNA nucleotides where a ribonucleoside is linked between the 2'-oxygen and the 4'-carbon atoms with a methylene unit. LNA oligonucleotides exhibit increased thermal stability towards complementary DNA and RNA with characteristically higher melting temperatures. *In situ* hybridization (ISH) utilizing LNA probes targeting rRNA sequences has not been described. This study details an ISH procedure using a biotin-labeled LNA probe targeting *Aspergillus sp*.18 S rRNA sequences.

Design: A genus specific 3' terminally biotin labeled oligonucletotide probe with the following sequence 5'-GCGGGTCATCATAGAAACACCGC-3' was commercially synthesized using a mixture of DNA (60%) and LNA (40%). ISH was performed on 16 culturally proven formalin-fixed, paraffin-embedded (FFPE) cases of *Aspergillus sp*. using modified capillary action technology on the Microprobe staining system. The tissues were dewaxed, cleared, rehydrated and then digested with 2.5 mg/ml of pepsin. The LNA probe was applied to the tissue sections and heated at 105°C briefly followed by hybridization for 1 hour at 50°C. The tissues were washed with 2XSSC and the hybrids were detected using anti-biotin conjugated to alkaline phosphatase followed by Permanent Red chromogen.

Results: A series of 16 FFPE tissues with culturally proven Aspergillus cases were studied using the ISH procedure. This included *A fumigatus* (12 cases), *A flavus* (2 cases), *A terreus* (1 case) and *Aspegillus sp*. not further specified (1 case). The probe effectively detected *Aspergillus sp.* rRNA sequences in all specimens. In addition, ISH with the Aspergillus specific probe was negative on culture proven cases of *Candida sp*, Mucor, Fusarium and *Scedosporium apiospermum*.

Conclusions: ISH using an LNA oligonucleotide probe targeting Aspergillus 18S rRNA sequences can be useful for detecting *Aspergillus sp*. in paraffin-emebedded tissue specimens. This test could be utilized when fungal pathogens are observed in tissue but cultures are negative or have not been performed. *In situ* hybridization with LNA probes may be useful for detecting a variety of fungal pathogens in FFPE specimens.

1309 Genital and Perianal Herpes Simplex Simulating Neoplasia in AIDS Patients

MB Mosunjac, J Park, W Wang, TS Tadros, MT Siddiqui, JH Sequeira, JV Little. Emory University, Grady Memorial Hospital and Crawford Long Hospital, Atlanta, GA.

Background: Perianal and genital infectious diseases are common in HIV-infected patients. Herpetic ulcer is considered a hallmark of AIDS, however HSV in combination with HIV may cause atypical clinical presentation simulating neoplastic process that can result in delayed diagnosis and treatment.

Design: 9 immunocompromised HIV positive patients with unusual tumoral presentation of anogenital herpes were identified. Laboratory data, clinical and histopathological findings were analyzed.

Results: There were 6 male and 3 female patients (37-53 years old), all with long standing AIDS history and all under higly active anti-retro-viral therapy (HAART). 6 patients presented with scrotal or vulvar masses and 3 with perianal nodules. 5 patients had adjacent HPV-related lesions. Herpetic lesion was clinically suspected in only 3 patients and in the rest of the patients a malignant growth was the main clinical concern. Predominant histopathological finding was dense dermal plasmacytic infiltration with overlying pseudoepitheliomatous hyperplasia, superficial ulcers and classic herpetic inclusions.

Conclusions: In conclusion, patients with AIDS may experience excessive number and size of both primary and reactivated herpetic lesions. Less frequent, but often clinically misdiagnosed are HSV lesions with tumoral, hypertrophic, nodular or verrucous growth characterized by pseudoepitheliomatous hyperplasia and dense plasma lymphocytic infiltration. It is important to be aware of such atypical presentation to avoid delay in correct diagnosis and treatment.

Clinical Presentation

Case	Site	Gross appearance	Largest dimension(cm)	HPV-related lesion	Clinical diagnosis
1	vulva	ulcerated and necrotic mass	2.0	Condyloma	Condyloma
2	scrotum	large scrotal mass with superficial ulceration	5.0	None	Tumor
3	perineum and scrotum	gray white, verrucous nodule	7.0	Condyloma	Condyloma and herpes
4	vulva perineum gluteal region	exophytic and nodular lesion with adjacent flat and granular areas	3.6	VIN III, AIN III/CIS	Condyloma, r/o icarcinoma
5	anus	nodular mass	2.7	AIN III and Condyloma	Condyloma r/o herpes
6	perianal region and scrotum	nodular tan mass	6.8	None	Tumor mass
7	perineum	ulcerated thickening and fissure	3.0	None	Condyloma, HIV-related infections
8	perianal region	tan nodular tumor	9.0	AIN II-III	Tumor, condyloma, r/o infiltrating process
9	scrotum	white nodules with erythematous ulcerations	1.9	None	Lymphoma vs herpes

1310 HIV Status and Anal Cytology: A VAMC Experience

T Mounajjed, HX Bui, JP Reynolds, S Samaan, VL Skeen, J Robertson, DM Moore, D Smulian. Veterans Affairs Medical Center, Cincinnati, OH; University of Cincinnati, Cincinnati, OH.

Background: In the HAART era, survival improvement for HIV patients is associated with high risk of anal intraepithelial neoplasia (AIN) and anal cancer. There is contradicting data regarding the relationship of CD4 count and viral load to AIN. The relationship of HIV mutations to AIN has not been reported.

Design: 263 anal smears from 142 HIV patients between March 2005 and March 2007 were independently reviewed by 2 investigators using Bethesda criteria. HPV genotyping was performed on abnormal smears (ASCUS or worse). 111 serum CD4 cell counts and 188 HIV viral loads (VL) were obtained with (<4 months before or after) the smear. We compared: 1. CD4 count and VL between negative smears and abnormal smears using a student t test. 2. CD4 count and VL between negative, ASCUS, ASC-H, LGSIL, and HGSIL smears using ANOVA test. 3. CD4 count and VL between patients with negative smears who later converted to abnormal smears (converters) and patients whose smears remained negative (non-converters) using a student t test. 4. CD4 count and VL between patients with high risk HPV (HR-HPV) and other patients. We also reviewed HIV mutations obtained by HIV genotyping in 58 patients and studied their relationship to smear abnormality.

Results: 1. VL was: A. Significantly higher in abnormal smears (mean: 28412 copies/ml) than in negative smears (mean: 1321 copies/ml) (p= 0.0246). B. Significantly higher in converters (mean: 10560 copies/ml) than non-converters (mean: 1754 copies/ml) (p=0.0178). 2. CD4 count: A. No statistically significant difference between negative smears (mean: 734.5) and abnormal smears (mean: 740). B. No statistically significant difference between converters and non-converters. 3. CD4 count and VL showed no correlation with the grade of smear abnormality. 4. Presence of HR-HPV was not associated with significantly different CD4 count or VL. 5. No mutation showed significant correlation with the presence or degree of abnormality. However, a higher prevalence of abnormal smears was seen in patients with of K70R (60%) and ML14V (56.3%) mutations than without mutations (14.3%).

Conclusions: 1. Higher VL in HIV patients was associated with the presence of anal dysplasia and with conversion from normal to abnormal smear. CD4 count showed no such association. 2. There was no association between CD4 count or VL and the degree of smear abnormality. 3. HIV genotype mutations showed no relationship to the presence or grade of smear abnormality. 4. Presence of HR-HPV showed no relationship to CD4 count or VL.

1311 Histologic, Immunohistochemistry, Microbiologic and Ultrastructural Characterization of Pulmonary Tularemia

P Navarro, M Garcia-Moliner, H Safaii, Z Berrada, S Telford, J Alroy. Tufts-New England Medical Center, Boston, MA; Tufts Veterinary School, Grafton, MA.

Background: Tularemia is a rare zoonosis caused by the gram negative bacterium *Francisella tularensis*. Although fewer than 150 cases are reported each year from the U.S., Martha's Vineyard, Massachusetts has experienced an outbreak for the last 7 years. Of the more than 60 confirmed cases from that island, nearly two thirds presented with pulmonary involvement suggestive of exposure by inhalation. Epidemiologic studies demonstrate at least a 6 fold greater risk for exposure during landscaping activities. A severe case of pulmonary tularemia was recently treated at Tufts-NEMC. We compared different diagnostic tools for confirming the etiology and characterizing the lesions of pulmonary tularemia.

Design: Pleural effusion was collected for microbiological study, paraffin sections from a lung biopsy were stained with H&E, CD3, CD68 and polyclonal chicken anti-tularemia IgY. Epon embedded sections were processed for electron microscopy.

Results: A 22 year old landscaper from Martha's Vineyard presented with fever, cough, pleuritic pain and shortness of breath. The patient was treated with gentamicin but severe signs and symptoms persisted. X-rays revealed consolidation of the left lower lung lobe. *Francisella tularensis* was cultured from the pleural fluid and serology demonstrated a 1:256 titer. A wedge biopsy of the left lung was obtained 2 weeks after his admission. Samples for light and electron microscopy were characterized by several foci of central necrosis surrounded by a mixed cell population of numerous enlarged vacuolated CD68 positive macrophages, CD3 positive T lymphocytes, neutrophils and proliferating fibroblasts. Electron microscopy revealed numerous vacuolated macrophages with lysosomes containing bacterial and cellular debris near the necrotic foci. Similarly,

IHC using a polyclonal anti-tularemia antibody demonstrated *F. tularensis* antigen within the cytoplasm of macrophages solely near the necrotic foci. The association of *F. tularensis* antigen and ultrastructural detection of bacterial remnants near the necrotic foci confirms the etiology of the central necrosis.
Conclusions: Establishing that *F. tularensis* caused the lesions that were observed is hindered by the fact that the agent stains poorly with typical histopathologic methods. The use of multiple histopathologic techniques facilitated the description of the lesions of severe pulmonary tularemia.

1312 HIV and Mast Cells in Human Tissue

AM Nelson, A Auerbach, Y-G Man. Armed Forces Institute of Pathology, Washington, DC.
Background: Mast cells have recently been identified as potential reservoirs of human immunodeficiency virus (HIV) by electron microscopy, flow cytometry and PCR. This has not been validated in HIV-infected human tissue.
Design: Paraffin-embedded lymphoid tissue from various sites (lymph node, cervix, salivary glands, and GI-tract) were retrieved from the files of our institution. All patients were known to be HIV-infected and none had mast-cell disease. Sections were subjected to double-labeling by immunohistochemical staining for p24 capsid protein of human HIV and mast cell tryptase using two different colored chromogens. Sections were evaluated to determine what direct association exists between HIV and mast cells. We looked for co-localization of both chromogens within the same cell and at the distribution of mast cells in proximity to p24+ cells.
Results: All 10 cases showed P24 positivity in individual cells or on surface of follicular dendritic cells. Tryptase positive (mast) cells were present in all cases. There was no visible co-localization of P24 protein in tryptase positive cells. P24 was localized in and in close proximity to the follicular dendritic network in nodal and extranodal tissues. Mast cells were not seen in the same areas as p24+ cells.
Conclusions: Although this study does not refute the potential of mast cells as a reservoir of HIV, we did not find immunohistochemical evidence of P24 in mast cells from patient material. Further investigation of this relationship is needed.

1313 *Bartonella* Endocarditis in Non-Immunocompromised Patients: Report of Four Cases

MD Oethinger, NK Shrestha, LJ Doyle, DA Wilson, AI Kim, TG Fraser, SM Gordon, GS Hall, CD Tan, ER Rodriguez. Cleveland Clinic, Cleveland; Cleveland Clinic, Cleveland, OH.
Background: Culture-negative infective endocarditis continues to be a diagnostic challenge for physicians. Molecular techniques have shown that *Bartonella* spp. are among the most frequent etiologic agents of culture-negative infective endocarditis, but prevalence varies highly between different geographical regions.
Design: Between 2003 and 2007, four cases of *Bartonella* endocarditis were identified at our institution by molecular methods directly from the heart valve (PCR assay with LightCycler® hybridization probe technology (Roche, Indianapolis, IN) and subsequent melt curve analysis). Clinical presentation and histopathologic features of the four patients were reviewed. Furthermore, we applied our *Bartonella*-specific PCR to a repository of frozen heart valves from patients with culture-negative infective endocarditis during the same time period.
Results: All four patients had prosthetic devices and had undergone at least one heart valve operation between 1 and 10 years prior to this episode. Histopathologic findings showed fibrinous vegetations with histiocytes, rare to few neutrophils and commonly microcalcifications. Granulation tissue with predominantly lymphocytic infiltrates was present in native valves. No further cases of *Bartonella* endocarditis were found by PCR in 30 patients with confirmed culture-negative infective endocarditis.
Conclusions: In our series from a large referral heart center, *Bartonella* endocarditis was exclusively seen in middle aged patients with prior heart valve surgeries. The disease presentations varied from acute to chronic infective endocarditis. A high degree of suspicion is necessary to initiate additional studies such as serology or PCR assays. Molecular diagnosis plays a definite role in elucidating the pathogen in patients with culture-negative infective endocarditis at time of surgery.

Demographic data and histopathologic findings in *Bartonella* endocarditis

Patient	Age/Sex	Valve	Histopathology	Warthin-Starry stain
1	53/M	Mitral valve annular ring prosthesis	Fibrinous vegetation, granulation tissue	Not done
2	56/F	Native mitral valve, aortic valve homograft	Fibrinous and organizing vegetations, granulation tissue	Not done
3	54/M	Bioprosthetic aortic valve	Fibrinous and organizing vegetations	Rare coccobacilli
4	63/M	Aortic valve homograft	Fibrinous and organizing vegetations	Rare pleomorphic bacteria

1314 Stratification Is Critical: Plasma Biomarkers Correctly Direct Therapy in Experimental Sepsis

MF Osuchowski, J Connett, K Welch, J Granger, DG Remick. Boston University, Boston, MA; U of Michigan, Ann Arbor, MI.
Background: Septic patients have an extremely high fatality rate and inhibition of the inflammatory response has not improved survival. Retrospective analyses of failed trials implied that anti-inflammatory interventions have potential benefits when targeting sub-populations with a high risk of death. Previous work has identified plasma biomarkers that predict mortality in the murine sepsis model induced by cecal ligation and puncture (CLP).
Design: In this study we examined whether non-specific inhibition of the inflammatory response with dexamethasone (DEX) would improve survival if appropriately targeted to mice with a high probability of death. After CLP, mice (n=88) were prospectively divided into 2 groups either predicted to die (die) or predicted to live (live) based on plasma levels of IL-6 or IL-1ra obtained 6 hours after CLP. DEX was injected intraperitoneally (2.5 mg/kg bw) at 8 and 32h post-CLP to 50% of animals from each group while the remaining animals received saline only.
Results: Without stratification, DEX did not bring about any improvement. Also, in those mice predicted to live, there was no difference in survival between the saline and DEX treatment. In the predicted to die group, DEX improved survival with 4/10 mice alive after 3 days of sepsis compared to 1/9 in the saline group. Mice were followed for 28 days, since it is possible that early DEX treatment would improve short term survival but compromise the host response so that they mice would fail to clear the infection. The survival advantage conferred by DEX persisted over the 28 days with 4/10 mice alive with DEX compared to 0/9 with saline (p=.012 by log rank survival analysis). Plasma cytokines 24 hours after sepsis (18 hours after DEX or saline) were measured to determine the mechanism of the improved survival, but this dose of DEX did not significantly alter the cytokine levels.
Conclusions: Prospective stratification based on plasma cytokines is feasible and accurate since DEX improved survival of mice predicted to die. The study also suggests that excess inflammation may cause the early septic deaths. When tailored, even a broad and non-specific anti-inflammatory immunosuppressive treatment may be more successful then a sophisticated drug used indiscriminately.

1315 Pattern of Blastomycosis in Surgical Pathology: Morphology Compared to Microbiologic Culture

A Patel, P Gattuso, V Reddy. Rush University Medical Center, Chicago, IL.
Background: Blastomycosis is a worldwide disease caused by inhalation of spores of the dimorphic fungus, *Blastomyces dermatitidis*. The diagnosis can be made by microbiologic culture or by morphologic identification of broad-based budding yeast forms in tissue samples. Given the paucity of studies comparing the sensitivity of cultures and morphology, a retrospective study was undertaken to compare both methods and to assess the anatomic distribution patterns and clinical features of blastomycosis.
Design: Review of the anatomic pathology records at Rush University Medical Center from January 1998 to April 2007 identified 53 patients diagnosed with blastomycosis. Of these, 37 were male and 16 female; age 14-77 yrs, mean 46. The following characteristics were reviewed: number of cases diagnosed per year, anatomic distribution of the disease, host factors such as immunosuppression or cancer, clinical presentation, correlation of histologic/cytologic diagnosis with microbiologic culture, and adverse outcomes.
Results: 52/53 (98%) patients were diagnosed with blastomycosis by histologic and/or cytologic examination while one (2%) was positive by culture but negative on tissue exam. 46 patients (87%) had concomitant cultures. Of these 32 (69.6%) were positive, 10 (21.7%) negative, and 4 (8%) showed other fungi. 29 cases (54.7%) involved lung, 13 (24.5%) soft tissue and bone, 4 (7.5%) skin, 3 (5.6%) other sites, and 3 (5.6%) occurred in more than one site. All patients except one presented with respiratory, bone, or skin related symptoms. 14 (26%) patients were immunosuppressed or had cancer. 11/32 (34%) patients with lung involvement were initially suspected to have a primary lung carcinoma. Five patients expired soon after diagnosis, 4 of whom had coexistent systemic illnesses. The number of cases diagnosed per year increased from 2 in 1998 to 6 in 2007.
Conclusions: 1. Blastomycosis encountered in surgical/cytopathology can be reliably diagnosed by morphologic examination. 2. Lung is the most common site of involvement followed by bone and soft tissue. In 34% of patients with lung involvement, the clinical diagnosis was neoplasm. 3. Majority of patients (98%) presented with clinical symptoms related to the site of involvement. 4. Morphologic diagnosis on tissue/cytology specimens appears to be more sensitive than microbiologic cultures, allowing prompt clinical management. 5. Based on our series, there appears to be an increase in the incidence of blastomycosis in the last 10-year period.

1316 Histologic Findings of Infections Caused by the Rapidly Growing *Mycobacterium* Species: Non-Specific Histologic Findings Complicate the Anatomic Diagnosis

PG Robinson, MC Reyes, GW Procop. Jackson Memorial Hospital/Miller School of Medicine at the University of Miami, Miami, FL.
Background: The rapidly growing *Mycobacteria* species are ubiquitous environmental organisms that may produce cutaneous infections in both immunocompromised and immunocompetent patients. The surgical pathologist receives a specimen labeled as debridement and sees granulation tissue, but fails to identify the mycobacteria. This abstract seeks to identify histologic clues as to the presence of *Mycobacterium* species.
Design: Mycobacterial cultures from patients that grew *M. chelonae*, *M. fortuitum* and *M. abscessus* from 2005 through 2007 were identified. The surgical pathology files were reviewed looking for tissue specimens. The corresponding specimens were reviewed with regard to the histologic changes and a Fite-Faraco stain was performed on a representative section from each specimen.
Results: Eleven cases of infection with either *M. fortuitum* or *M. abscessus* were identified with eight specimens being cutaneous and three from bone. On seven of the specimens, acid fast organisms could be identified on the Fite-Faraco stain. The hematoxylin and eosin sections were fairly similar from one specimen to the other. The cutaneous tissue showed ulcerated skin with scarring of the dermis. Adjacent to the scarred dermis was a loose, myxoid granulation tissue with focal collections of neutrophils. The granulation tissue contained a mixture of neutrophils, lymphocytes and plasma cells. Occasional multinucleated giant cells were present, but there was no distinct granuloma formation. One case of *M. abscessus* showed microbubbles in the granulation tissue with numerous organisms in the microbubble. The bone marrow showed a similar picture, but also contained areas of dense fibrosis. Again, there were no granulomas.

Conclusions: Cutaneous and osseous infections by either *M. fortuitum* or *M. abscessus* show acute and chronic inflammation in a background of loose granulation tissue with an occasional multinucleated giant cell. In order for a pathologist to consider a mycobacterial infection, they generally expect granulomas to be present. In the rapidly growing mycobacterial infections, granulomas are not formed and hence the pathologist may not consider the possibility of a mycobacterial infection. In one specimen, there was a distinctive microbubble with acid fast organisms present. The microbubbles may be a clue to the presence of mycobacteria. Correlation between the clinical microbiologist and the anatomic pathologist is essential.

1317 Does PCR for Cytomegalovirus (CMV) Performed on Gastrointestinal / Liver Biopsies Aid in the Diagnosis of CMV Infection?

TC Rubinas, MB Miller, HH Herfarth, ES Dellon. UNC Chapel Hill, Chapel Hill, NC.

Background: Microscopic evaluation of gastrointestinal / liver biopsies plays an important role in the detection of CMV infection. Performance of qualitative CMV RT-PCR on biopsy tissue has led to discordant results with histologic findings. We sought to investigate the role tissue biopsy RT-PCR plays in the diagnosis of GI / liver CMV infection.

Design: GI / liver biopsies with positive results for tissue qualitative CMV RT-PCR were retrospectively identified. We compared the positive tissue PCR results with the histologic findings of CMV viral cytopathic effect and available blood quantitative CMV PCR results.

Results: Eighteen biopsies had CMV detected by qualitative RT-PCR performed on biopsy tissue (5 esophagus, 2 liver, 2 small intestine, 9 colon). Six (33%) of the cases had concordance between histology and tissue CMV PCR. Three of these 6 had concomitant blood quantitative CMV PCR performed and CMV DNA was detected in each case (range: á500 to ñ1,000,000 copies/mL). Twelve (67%) cases demonstrated discordance between histology and tissue PCR. Six of these 12 had concomitant blood PCR performed, of which 4 had detectable CMV DNA (range: á500 to 2745 copies/mL). The remaining 2 cases had no detectable CMV DNA in the blood. Immunohistochemistry for CMV early antigen was performed in 5 of the 18 cases and was negative in each case. Two of the 5 were negative by both histology and blood PCR; 1 was negative by histology with á500copies/mL by blood PCR; 1 was positive by histology with 1157 copies/mL by blood PCR, and 1 was negative by histology and blood PCR was not done.

Conclusions: Tissue CMV qualitative RT-PCR may provide high false positive results while histologic examination, with or without immunohistochemical studies, may not be sensitive enough in the identification of CMV infection in biopsy specimens from the gastrointestinal tract and liver. Additional studies need to elucidate the role tissue CMV PCR has in the diagnosis of CMV infection from these sites. A combination of diagnostic tests (histology and PCR) may become the gold standard for the detection of CMV infection in the GI tract and liver.

1318 *Aspergillus* and *Candida* Immunohistochemistry of Culture-Confirmed Fungal Tissue Isolates Show High Cross-Reactivity

AN Schuetz, C Cohen. Weill Cornell Medical College, New York, NY; Emory University School of Medicine, Atlanta, GA.

Background: Increased use of immunosuppressive drugs has broadened the range and increased the incidence of invasive fungal diseases. Invasive aspergillosis and zygomycosis cause high morbidity and mortality in the immunosuppressed population. Successful therapy relies on early diagnosis, and antifungal susceptibilities differ among fungi. Special stains of fixed tissue may aid in fungal identification; however, *Pseudallescheria boydii*, *Fusarium*, and sometimes yeast, can resemble *Aspergillus* on histopathology, especially in necrotic areas. Cultures are not always reliable, especially for the zygomycetes. Since commercially available fungal immunohistochemistry (IHC) has been validated for cross-reactivity with few other fungi, we ran *Aspergillus* and *Candida* IHC on fixed tissue of culture-confirmed fungal cases.

Design: Commercially available *Aspergillus* and *Candida* IHCs were run with antigen retrieval on 15 culture-confirmed fungal cases (8 *Aspergillus*, 3 *Candida*, 2 *Bipolaris*, 1 *Fusarium*, 1 *Curvularia*). The polyclonal *Aspergillus* antibody (Biocare Medical, Concord, CA) raised against a soluble extract of *Aspergillus fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* and the monoclonal *Candida albicans* antibody (Chemicon International, Temecula, CA) had not been tested for cross-reactivity against other fungi. Three additional cases which stained positive for Zygomycete IHC at the Centers for Disease Control and Prevention (CDC) which had not been confirmed by culture were also tested against these two antibodies.

Results: Fourteen of the 15 culture-confirmed cases stained with *Aspergillus*, and two stained with *Candida*. All *Aspergillus* cultures (8/8) were correctly identified on IHC (sensitivity 100%). The six false positive *Aspergillus* IHCs (2 *Candida*, 1 *Fusarium*, 3 dematiaceous fungi) led to a positive predictive value of 57%. Both *Candida* IHC were culture-positive (specificity 100%); one, also positive for *Aspergillus*, showed greater *Candida* reactivity. One of the three Zygomycete IHC cases from the CDC stained weakly positive for *Candida,* and the other two were negative for both fungi.

Conclusions: Although sensitivity of *Aspergillus* IHC was high, there was high cross-reactivity with other fungi. *Candida* IHC showed good specificity, but the number of culture-confirmed cases was too low to adequately evaluate sensitivity. More accurate and reliable fungal IHCs, including zygomycete staining, are needed.

1319 Pathologic Studies of Rift Valley Fever from an Outbreak in Eastern Africa, 2006 – 2007

WJ Shieh, C Paddock, E Lederman, P Bloland, C Rao, H Gould, K Njenga, M Mohamed, D Kimanga, D Mutonga, S Amwayi, S Nichol, R Breiman, TG Ksiazek, SR Zaki. Centers for Disease Control and Prevention, Atlanta, GA; Centers for Disease Control and Prevention (US), Nairobi, Kenya; Ministry of Health, Nairobi, Kenya; Ministry of Tanzania, Dar es Salaam, Tanzania, United Republic of.

Background: Rift Valley fever (RVF) is an acute viral zoonotic febrile illness that affects domestic animals and humans. The disease is caused by the RVF virus, a member of the genus Phlebovirus in the family Bunyaviridae. RVF is endemic in regions of eastern and southern Africa. In December 2006, the Kenya Ministry of Health received reports of unexplained fatalities associated with fever and generalized bleeding from North Eastern Province. The outbreak was confirmed by isolation of RVF virus from clinical samples of affected patients and animals. As of March 12, 2007, a total of 684 cases of RVF had been reported in Kenya with 155 deaths.

Design: Postmortem tissue samples from 20 human cases with clinical suspicion of RVF were studied at CDC by using histopathologic examination and immunohistochemical (IHC) assays. The antibodies used in IHC assays included a polyclonal rabbit anti-RVF virus antibody and a mouse anti-RVF virus antibody. Other IHC tests were performed when indicated by compatible histopathologic findings.

Results: Extensive hepatocellular necrosis was identified in 12 of the 20 cases examined. Immunohistochemical evidence of RVF virus was present in 11 of these 12 cases. Immunostaining of viral antigens was observed mainly in necrotic hepatocytes and Kupffer cells of all 11 cases and in focal renal tubular epithelial cells of one case with a kidney sample available for testing. The only case with hepatocellular necrosis that was negative for RVF virus was subsequently identified as infection with human herpes simplex virus by IHC. Plasmodium falciparum was detected in the red blood cells of one case that had only a skeletal muscle available for testing.

Conclusions: RVF is among the most important viral zoonoses in Africa. Our studies show that extensive hepatocellular necrosis is the most prominent histopathologic change in liver of RVF. Similar change can be seen in hemorrhagic fever caused by other viruses. IHC methods is a useful diagnostic method for fatal cases with a clinical suspicion of RVF. Pathologic studies on postmortem tissue samples can also help establish the diagnosis of other infections, especially when clinical manifestations of those infections are similar to RVF.

1320 Multi-Drug Resistant Acinetobacter Infections in Rehabilitated Veterans of Operation Iraqi Freedom

LB Thomas, MJ Kasper, B Neugaard, RL Oehler, SG Scott, AA Bokowski, AV Heal, JN Burgett, SM Mastorides. University of South Florida, Tampa, FL; James A Haley Veterans Hospital, Tampa, FL; National Naval Medical Center, Bethesda, MD.

Background: Multidrug-resistant *Acinetobacter* (MDR-Acb) is frequently encountered colonizing or infecting veterans of Operation Iraqi Freedom (OIF). We characterize these infections in veterans treated at the poly-trauma and spinal cord injury rehabilitation centers at the James A. Haley Veterans Hospital.

Design: Ninety-one casualties of OIF were admitted to our rehabilitation center through October, 2006. Patient demographics and culture results were examined by electronic and paper chart review from federal healthcare facilities in the US and Germany before, during, and after their stay at our institution. Resistance patterns to 7 classes of antibiotics were reviewed. MDR was defined as resistance to ≥4 classes of antibiotics. In addition, internal chart review was conducted on non-OIF veterans with Acb infections treated at our facility both before and after the first OIF veteran arrived.

Results: The most common cause of injuries (62%) were from improvised explosive devices (IEDs) or other explosions, with head or extremity injuries being most frequent. Of OIF veterans, 56% had cultures positive for Acb, with 85% of these being MDR and 25% developing infections resistant to all antibiotics tested. In decreasing order, positive cultures were from the skin, respiratory tract, wounds, urinary tract, bloodstream, and rarely other sites. Average length of hospitalization and rehabilitation were longer in patients with Acb cultures (128.6 d) compared to those without Acb (89.2 d)(p=0.05). Four patients with Acb cultures died, while all patients without Acb cultures were alive at the end of the study period. Furthermore, review of hospital acquired Acb infections revealed that the average prevalence of MDR in non-OIF inpatients increased from approximately 3% to 20% after arrival of OIF veterans to our institution.

Conclusions: MDR-Acb colonizations and infections are a common problem in OIF casualties, with over half of the patients in our study having positive cultures at some point in there recovery. Patients with Acb infections have longer hospitalizations and have higher mortality, although the later was not statistically significant. Our data as well as that of others suggests that these infections readily spread to non-OIF hospitalized patients as well.

1321 Glycosylphosphatidylinositols: A Potential Target for New Antifungal Agents

B Yan, B Taron, J Wang, P Orlean. University of Chicago Hospitals, Chicago, IL; University of Illinois, Urbana-Champaign, IL.

Background: The antibiotics used to treat fungal infections are limited to amphotericin B, nystatin and the azole family of compounds. With more patients receiving organ transplants and immunosuppressive regimens for systemic inflammatory and autoimmune disorders, as well as more patients suffering from AIDS, the number of immunocompromised patients is increasing. An adverse consequence of the increased use of antifungal agents is a higher incidence of antibiotic resistance. The paucity of effective antifungal drugs, as well as increasing resistance to these agents, has led to significant efforts to develop a wider range of drugs that target fungal organisms.

Design: Glycosylphosphatidylinositol (GPI) anchors, which are glycophospholipids that serve to attach certain soluble proteins to the external leaflet of the plasma membrane, represent an attractive target for the development of new antifungal compounds.

Although they are present in all eukaryotes, GPIs are essential in fungi for their role in crosslinking of the fungal cell wall. Previous work has shown that the GPI biosynthetic pathways are different in fungi and humans. Moreover, the fungal and human GPI biosynthetic pathways are regulated by different mechanisms. Previous research in our laboratory has shown that the Ras protein inhibits the initial enzyme in yeast GPI biosynthesis. We set out to identify new yeast mutants defective in GPI assembly using a colony sectoring screen. We then tested the ability of these mutants to carry out the first step of GPI assembly: the transfer of *N*-acetylglucosamine from the donor molecule UDP-GlcNAc to the acceptor lipid phosphatidylinositol to form the reaction product GlcNAc-PI.

Results: We identified the *Saccharomyces cerevisiae* mutant *swi1* in a colony sectoring screen for mutations synthetically lethal with *gpi1*, a mutation affecting the first step of GPI biosynthesis. We show that yeast *swi1* mutants are unable to efficiently execute the first and committed step of the GPI biosynthetic pathway, demonstrating that the Swi1 protein is required for effective synthesis of GPI anchors. We also demonstrate that *swi1* cells, which are defective in their ability to synthesize inositol, are unable to form GlcNAc-PI even when supplemented with excess inositol.

Conclusions: The identification of the Swi1 protein as an enhancer of GPI synthesis in yeast, but not humans, suggests divergent regulatory mechanisms that may be targeted for the development of new antifungal agents.

Kidney

1322 Multinucleated Giant Cells in Anti-Glomerular Basement Membrane Disease (AGBM)

M Abdelbaqi, CL Phillips, PW Walker, SM Bonsib. Louisiana State University Health Sciences Center, Shreveport, LA; Indiana University, Indianapolis, IN; Nephropathology Associates, Little Rock, AR.

Background: Inflammatory cells are numerous in crescentic forms of glomerular disease. Multinucleated giant cells (MGC), however, are uncommon but have been described in several case reports of AGBM disease and ANCA/systemic vasculitis-associated crescentic glomerulonephritis (GN).

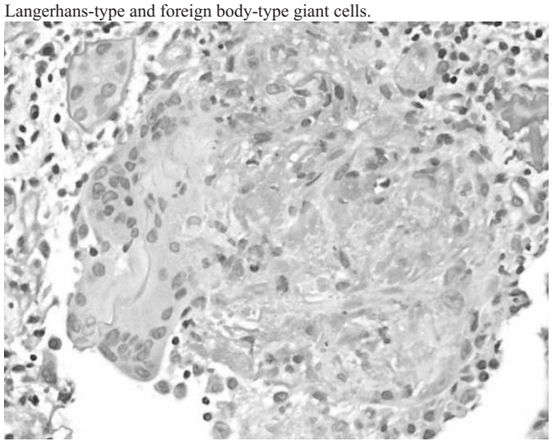
Design: Twenty-six cases of AGBM disease and 50 cases of non-AGBM crescentic GN (32 pauci-immune; 12 systemic lupus erythematosus, 6 immune complex-associated) were reviewed and the percentage of glomeruli containing MGC were determined.

Results: 18/26 cases of AGBM contained MGC (69%), compared with 2/50 non-AGBM diseases (4%). In the 2 non-AGBM cases with MGC, 1 MGC was noted in a single glomerulus in each case. In AGBM disease the percentage of glomeruli containing MGC ranged from 10-100% (see table).

Multinucleated Giant Cells in Crescentic GN

			% of Crescentic Glomeruli with MGC		
Disease	# Cases	# with MGC	>10%	>20%	>40%
AGBM	26	18	18 cases	15 cases	5 cases
Non-AGBM	50	2	0	0	0

Affected glomeruli ranged from acute necrotizing lesions with cellular crescents to fibrocellular and fibrous crescents. MGC were identified in completely sclerotic glomeruli in 6 cases. One case had 1 interstitial focus of MGC not clearly related to a glomerulus. The number of MGC per glomerulus ranged from 1 to 4 and included Langerhans-type and foreign body-type giant cells.



No basement membrane or matrix remnants were detected within the MGC on PAS or silver stains. Immunoperoxidase stain for CD68 was positive and cytokeratin was negative in the MGC in the cases tested.

Conclusions: (1) MGC are common in AGBM and rare in non-AGBM crescentic diseases (2) The presence of MGC strongly supports a diagnosis of AGBM in the absence of serologic and immunofluorescence data (3) Formation of MGC in AGBM likely reflects the immunopathogenesis of AGBM disease rather than the presence of a necrotizing event since MGC are usually absent in other crescentic diseases.

1323 Prognostic Pathological Features in Renal Biopsies in Scleroderma Renal Crisis

I Batal, R Domsic, T Medsger, Jr., L Kiss, P Randhawa, S Bastacky. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Scleroderma renal crisis (SRC) is an idiopathic microangiopathic disorder that affects the kidneys in a subset of patients (pts) with systemic sclerosis. Aggressive therapy with ACE inhibitors may prevent permanent renal failure in some pts. This study was designed to identify pathological prognostic factors and to investigate the role of a C4d in SRC.

Design: We retrospectively reviewed the pathology and the clinical records of 17 pts that underwent kidney biopsies during SRC at the University of Pittsburgh Medical Center between 1990 and 2007. Multiple histological features were assessed semi-quantitatively (0-3+) or as percentages. C4d staining of peritubular capillaries (PTC) and arteries was assessed in SRC pts (n=10) and controls (n=8) using a semi-quantitative scoring system (0-3+). Pts recovering renal function (group A: n=7) and those remaining in renal failure or dying (group B: n=10) were compared.

Results: No difference was found comparing age, sex, or presenting serum creatinine, however, a trend toward a difference in diastolic blood pressure (116 ± 13 mmHg group A vs 99 ± 21 mmHg group B) was noted. The percentage of thrombosed arteries/infarction was significantly higher in group B (28.5 ± 19.2) than group A (5.6 ± 12.3; p=0.017), as well as glomerular ischemic collapse (2.9 ± 0.3 group B vs 1.43 ± 0.79 group A; p=0.001). Also, group B pts showed a trend toward more severe acute tubular injury (1.8 ± 0.63 vs 1.14 ± 0.69; p=0.1) and fibrinoid necrosis (0.7 ± 0.95 vs 0 ± 0; p=0.195). Other histologic parameters assessed were similar between the two groups. C4d score was higher within arteries in scleroderma pts (2.11 ± 1.27) compared with controls (0.5 ± 0.9; p=0.023), and trended to be higher within PTCs [1.2 ± 1 scleroderma vs 0.25 ± 0.7 controls; p=0.055]. Moreover, C4d score trended to be higher in group B within PTC (1.67 ± 0.81 group B vs 0.5 ± 1 group A, p=0.11) as well as arteries (2.8 ± 0.5 group B vs 1.25 ± 1.5 group A; p=0.11).

Conclusions: The presence of arteriolar thrombosis/infarction and severe glomerular collapse in SRC renal biopsies correlates with increased risk of failure to recover renal function and death. C4d staining was more frequently detected in both PTC and arteries in SRC pts compared to controls, suggesting an ongoing antibody-mediated injury, although no immune complex deposits were identified. C4d staining tended to be associated with a worse prognosis, however, a larger study is needed to more conclusively address this issue.

1324 Thymosin β4 (Tβ4), a Marker and Potential Mediator of Progressive Sclerosis, Is Increased in Chronic Allograft Nephropathy (CAN)

B Can, E Donnert, P Revelo, C Federspiel, AB Fogo. Vanderbilt University Medical Center, Nashville, TN.

Background: Tβ4 is a G-actin-sequestering protein with myriad functions, including cell differentiation, angiogenesis, wound healing, and regulation of inflammation. We have identified upregulated Tβ4 by proteomic analysis in a rat model of focal segmental glomerulosclerosis, and as a necessary intermediary for angiotensin II-induced upregulation of plasminogen activator inhibitor (PAI-1). PAI-1 inhibits both fibrinolysis and proteolysis and is linked to progressive scarring. We have shown previously that PAI-1 is increased in CAN (Revelo et al, NDT 2005). We therefore aimed to investigate whether Tβ4 is also increased in these cases of CAN, and is linked to sites of PAI-1 expression.

Design: 97 renal transplant biopsies or nephrectomy specimens from 75 patients with CAN were studied and compared with transplant (Tx) biopsies without CAN (n=8) and normal native kidney (n=7). Tβ4 expression and macrophage localization were detected by immunohistochemistry. Glomerular cell, tubular, interstitial, and vascular staining was scored as cells/glomerulus or on a 0 - 3 scale.

Results: Native and normal Tx kidneys showed Tβ4 staining only in collecting duct epithelial cells, rare peritubular capillaries and rare glomerular macrophages. In CAN, podocytes, non-podocyte glomerular cells, and parietal epithelial cells showed increased Tβ4 vs normal and normal Tx. Tubular Tβ4 staining was 0.92±0.48 and vascular staining was 12.9±16.9% in CAN and minimal in normal and native and normal Tx. Interstitial Tβ4 was dramatically increased in CAN, 2.69±0.47, correlating with fibrosis, and was absent in normal native and normal Tx. Interstitial and glomerular Tβ4 were both contributed to by infiltrating macrophages. Increased Tβ4 was present in particular in biopsies with increased PAI-1.

Conclusions: Tβ4 immunostaining is significantly increased in glomeruli, interstitium and vessels in CAN compared to native and transplant control kidneys, and is linked to fibrosis and PAI-1 expression. We speculate that Tβ4 contributes to fibrosis at least in part by augmenting PAI-1.

1325 In-Situ B Cell Immune Responses Contribute to the Pathogenesis of Human Lupus Nephritis

A Chang, S Henderson, N Liu, R Guttikonda, C Hsieh, SM Meehan, RJ Quigg, MR Clark. University of Chicago Medical Center, Chicago, IL.

Background: We have previously demonstrated significant quantities of interstitial B lymphocytes and plasma cells (PC) in human lupus nephritis (LN) kidneys. We hypothesize that analyzing the immunoglobulin (Ig) repertoire from interstitial PCs of LN kidneys may give us additional insight into this autoimmune disease.

Design: 70 kidney biopsies from LN patients were analyzed. The interstitial inflammation was divided into the following patterns: 1) diffuse and scattered; 2) T:B cell aggregates, or 3) germinal center (GC) formation, which was correlated with the presence of tubular basement membrane (TBM) immune complex deposition. The frozen tissue from a GC and another biopsy with T:B cell aggregates were identified. The sections were immunohistochemically stained with anti-CD38 and individual and small groups of PCs were isolated by laser capture microdissection (LCM) using the Arcturus Pixcell II system. RT-PCR was performed directly on the LCM caps and IgG primers to the heavy and light chains were used. The products were sequenced and the antibody sequences were analyzed. The germline sequences were identified by aligning sequences in IMGT/V-QUEST.

Results: Six of 33 (18%) biopsies with the diffuse and scattered inflammatory pattern compared with 21 of 37 (57%) biopsies with either T:B cell aggregates or GCs contained TBM immune complex deposition (p=0.0006). Sequence analysis of Ig expression of