1614 Negative TdT Expression Predicts Adverse Treatment Outcome in T-Lymphoblastic Leukemia/Lymphoma in Adults

Y Zhou, M Routbort, KH Young, S Wang, D Hoehn, G Tang, C Bueso-Ramos, CC Yin, RN Miranda, LJ Medeiros, P Lin. The University of Texas, MD Anderson Cancer Center, Houston, TX.

Background: T-lymphoblastic leukemia/lymphoma (T-ALL/LBL) is an aggressive disease that requires intensive chemotherapy regimens such as hyperfractionated cyclophosphamide, vincristine, adriamycin, and dexamethasone (Hyper-CVAD). With this therapy, approximately 90% of the patients initially achieve complete remission. Nevertheless, a significant subset of patients relapses and succumbs to recurrent disease. Unlike patients with B-ALL/LBL, there is currently no risk stratification scheme for T-ALL/LBL patients. To identify the high-risk patients who may benefit from stem cell transplant during the first remission is clinically challenging. A readily accessible prognostic marker for high-risk T-ALL/LBL is essential for risk-adapted therapy and improved outcome.

Design: We reviewed available data of T-ALL/LBL patients treated at our institution between 2003 and 2011 (n=106), and identified TdT negative cases. Negative TdT immunoreactivity was defined as <10% neoplastic cells positive by combined flow cytometric immunophenotyping and immunohistochemical staining. All cases were positive for cytoplasmic CD3. Clinical, morphologic, immunophenotypic and cytogenetic data were reviewed. Relapse-free survival and overall survival were calculated using Kaplan-Meier survival analysis.

Results: We identified 17 (16%) cases of TdT-negative T-ALL/LBL: 8 *de novo* and 9 relapsed. There were 12 men and 5 women with a median age of 30 years (range, 13 to 62). The median follow-up period was 12 mo (range, 2.9 to 42.3 mo). The immunophenotype was pro-T/pre-T (CD34+) in 8 neoplasms, cortical-T (CD1+) in 5, or other (CD34-/CD1-/CD4-/CD8-) in 4. Of 16 cases with cytogenetic data, 11 had a complex karyotype, 4 were diploid, and 1 had isolated add(3). All patients received intensive chemotherapy, including hyper-CVAD, as frontline or salvage treatment. The estimated relapse-free and overall survival at 2 years was 8 % and 24%, respectively. Among the 8 *de novo* patients, the estimated relapse-free and overall survival at 1 year was about 24%. Relapse-free and overall survival in this patient group was significantly worse than those of age-matched TdT positive T-ALL/LBL patients treated during the same period ($p<0.01$).

Conclusions: About 15% of patients with T-ALL/LBL are negative for TdT immunoreactivity, and respond poorly to intensive chemotherapy. Stem cell transplant during first remission may be beneficial for this group of patients.

Infections

1615 A Comprehensive Study of Whipple Disease: Diagnostic Clues from Unusual Presentations

C Arnold, R Moreira, E Montgomery. Johns Hopkins Hospital, Baltimore; Columbia University College of Physicians and Surgeons, New York City.

Background: Although Whipple Disease was described over a century ago, *T. whipplei* remains difficult to culture and to eradicate. Timely diagnosis of this sometimes lethal disease is critical. We report cases with unusual clinical presentations and unusually subtle histology.

Design: All cases of Whipple Disease diagnosed since the introduction of the Whipple IHC (2002) were identified and the clinicopathologic information was obtained.

Results: Twenty one lesions were identified from 14 patients: the age ranged from 39 to 69 years (mean, median 53 years) and the study included 10 males (71%). Lesions involved small intestine (13, 61%), brain (2, 10%), heart valves (4, 19%), breast (1, 5%), and retroperitoneal soft tissues (1, 5%). Patients presented with site-specific complaints. Most small bowel biopsies (10/13) were from newly diagnosed patients and showed the classic features of Whipple Disease: dilated lacteals and lamina propria expansion by foamy macrophages containing PAS+/IHC+ granules. Three cases showed treatment effect: only occasional macrophages minimally expanding the lamina propria and rare PAS+/IHC+ material. Foamy macrophages containing PAS+/IHC+ material were also identified in the breast, cardiac, and brain biopsies. Follow-up data were available in 7 (50%) cases, and persistent disease noted in 6 (86%) cases. The latency period between onset of symptoms and a diagnosis of Whipple Disease ranged from a few to greater than 10 years. Alternative submitted clinical impressions included celiac disease, Crohn vasculitis, sepsis, inflammatory process, and liposarcoma. Limited treatment data featured IV ceftriaxone, compliance issues, and adverse side-effects. One patient died (7%), but complete follow-up information was unavailable.

Conclusions: We report unusual clinical presentations of Whipple Disease, including presentation as a breast and retroperitoneal masses. We also report cases of Whipple Disease with unusual histology, namely cases with treatment effect. Despite great advances over the past 100 years, Whipple Disease remains a great mimicker and is often misdiagnosed. In our series, the latency period between onset of symptoms and diagnosis was at least several years and submitted clinical impressions included celiac disease, Crohn vasculitis, sepsis, inflammatory process, and liposarcoma. Awareness of unusual presentations and subtle histology together with the appropriate utilization of the Whipple IHC is essential for recognition of this sometimes lethal condition.

1616 Fatal Leclercia Adecarboxylata Infection in an Immunocompetent Child: A Case Report and Literature Review

EM Barker, LA Metlay. University of Rochester, Rochester, NY.

Background: *Leclercia adecarboxylata* is a motile Gram negative rod initially described by Leclerc in 1962 as *Escherichia adecarboxylata*. It is a member of the Enterobacteriacae and is also formerly known as enteric group 41. The organism has been isolated from human stool, is considered a natural part of enteric flora, and occurs in the environment. Cases of infection by *L. adecarboxylata* are rare but usually occur in patients with immunocompromise or significant medical comorbidities and typically occur in a polymicrobial infection. In a review of the literature, only one other case of a fatality from *L. adecarboxylata* infection was found.

Design: We present the fatal case of a 5 year old boy with chronic colonic dysmotility who died from complications of *L. adecarboxylata* sepsis. We then present a synopsis of current available literature regarding *L. adecarboxylata* infections.

Results: The patient was a five year old boy who suffered from a chronic colonic dysmotility syndrome which caused severe constipation. For this condition he had undergone ileostomy two years prior, leaving the colon in place as a blind loop. A central catheter for parenteral nutrition was also in place. While at home in his usual state of health the boy developed several days of low grade fever and then suffered a seizure. He was taken to the hospital and found to have had an intracerebral hemorrhage secondary to disseminated intravascular coagulation caused by sepsis. The patient died before emergency surgery could be performed. *L. adecarboxylata* was grown in pure culture from a central line blood sample. The same organism grew from a blood sample collected at autopsy. Culture of the central catheter tip was negative. Although there are reports of some drug resistant strains of *L. adecarboxylata*, the organism isolated in this case was susceptible to all antibiotics tested. Possible routes of organism entry in this case include the central catheter and the blind loop of colon (which was found to contain stool at autopsy).

Review of the literature revealed 29 reported cases of infection by *L. adecarboxylata* with only one fatality. That patient was a 71 year old man with comorbidities of hepatocellular carcinoma and liver cirrhosis due to hepatitis C viral infection.

Conclusions: *L. adecarboxylata* is a rare but clinically significant organism that may cause fatal infection in humans. When isolated, the organism is often pan-susceptible to the antibiotic panels tested. Routes of entry may include central lines and entry through the gut.

1617 Merkel Cell Polyomavirus (MCPyV) Detected in Plasma of Post-Bone Marrow Transplant Patients by SYBR Green-Based Real-Time PCR and Melting Curve Analysis

SS Chen, JJ Tarrand, V Prieto, P Lin, MH Fernandez, T Hasan, LJ Medeiros, C Bueso-Ramos. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The novel Merkel cell polyomavirus (MCPyV) was first identified in Merkel cell carcinoma (MCC) in 2008. MCPyV is detected at high frequency (~80%) in MCC and is also reported in chronic lymphocytic leukemia (CLL). Post-bone marrow transplant (post-BMT) patients are immunosuppressed and frequently have reactivation of normally latent viruses, such as Epstein-Barr virus (EBV) and cytomegalovirus (CMV). There are no reports on detection of MCPyV in post-BMT patients, most likely due to the short time since the first discovery of this virus. Additionally, there are no reports of using plasma samples for detection of MCPyV.

Design: We developed a SYBR Green based real-time PCR and melting curve analysis method to detect MCPyV and we tested plasma samples. We initially developed the method using DNA from a MCPyV-positive cell line (MS-1) and validated the method on clinical samples of MCC (n=5) and CLL (n=2). Subsequently, we tested plasma samples obtained from post-BMT patients that had tested positive for EBV (n=10) or CMV (n=10) as part of routine laboratory workup.

Results: Using MS-1 cell line DNA, MCPyV was detected by SYBR Green-based real time-PCR and melting curve analysis generating a melting temperature peak of 81.7 +/- 0.2 ºC. The MCPyV PCR amplicon was confirmed by agarose gel electrophoresis producing the expected amplicon size as well as by DNA sequencing. By serial dilution of MS-1 DNA, the dynamic range and the limit of detection of the real-time PCR assay was established as 5-$\log_{10}$ and 0.16ng DNA, respectively. The analytical performance of the assay was tested on clinical samples with results as follows: 5 of 5 (100%) MCC formalin-fixed paraffin-embedded tissue (FFPET) samples and 1 of 2 CLL peripheral blood samples were positive for MCPyV. Out of 20 post-BMT plasma samples that were previously tested positive for EBV or CMV, MCPyV was detected in 5 (25%): 3 of 10 EBV-positive samples and 2 of 10 CMV-positive samples.

Conclusions: We report a SYBR Green-based real-time PCR and melting curve analysis method for detection of the novel MCPyV and show, for the first time, that this virus can be detected in plasma samples of post-BMT patients. The clinical importance of MCPyV in plasma samples needs further clinical and epidemiological investigation, for which the developed assay can be useful.

1618 Primary Hepatic Lymphoma in HIV Positive Patients Diagnosed by Image-Guided Fine Needle Aspiration: Clinico-Pathologic Correlation

M Deliu, ML Nguyen, CJ Gunthel, MB Mosunjac. Emory University, Atlanta, GA.

Background: The incidence of primary hepatic lymphoma (PHL) is rare and unexpected. However, recent studies show that it is increasing, particularly in HIV patients. Diagnosis of PHL is difficult not only due to the risks of liver biopsy, but also due to the array of ancillary studies needed for definitive diagnosis. The use of image guided fine needle aspiration (IGFNA) is proven to be safer and also allows obtaining adequate material for ancillary studies. Here, we report on clinico-pathologic characteristics of patients with PHL and the utility of IGFNA.

Design: A retrospective search (1996-2011) was conducted at the inner city hospital to identify 16 cases of PHL diagnosed by IGFNA. IGFNA was conducted by 19 or 20-gauge needles, performing an average of 3 passes.Pathologist was always present for immediate evaluation. Pathology slides and ancillary studies, as well as flow cytometry reports, were reviewed to reconfirm primary diagnosis. Demographic data (race, age, sex), laboratory data (HIV status, and HIV related lab tests, LDH), imaging data (CT), and clinical data were obtained from electronic patient charts.

Results:

Table 1. Patient characteristics (n=16) FNA

Age (median)	45
range	(30-69)
Male:Female	15:1
Race	12:4
African American: non-African American	
HIV +/-	15:1
CD4 count, average (range)	111 (7-291), n=15
Viral load, average (range)	370,000 (2000-750,000), n=3
HAART, n (%)	4 (24)
Clinical presentation, n	
Abdominal pain	7
Hepatomegaly	3
Jaundice	1
Other	2
CT of abdomen, n	
Solitary lesion	1
Multiple lesions	15
LDH, average (range)	1148 (283-3688), n=11

The most common types of PHL were: B cell lymphomas (12 of 16) including diffuse large B cell lymphoma (DLBCL) (n=6), B cell lymphoma NOS (n=4), follicular B cell lymphoma (n=1) and Burkitt's lymphoma (n=1). There was 1 case of T cell lymphoma and 1 case of Hodgkin's lymphoma. Only 2 cases failed to reach a definitive diagnosis of lymphoma either due to insufficient material or extensive cell necrosis. Imaging showed, predominantly, multiple lesions ranging from 1.7-12.3cm. 15 patients, (94%) were HIV positive, with AIDS defining CD4 counts. None were on HAART therapy, and no unifying risk factors were identified. 8 patients were recently diagnosed with HIV, whereas 4 patients were long standing.

Conclusions: In the population of an inner city hospital, PHL is primarily seen in AIDS patients. Most of the lesions were B-cell lymphomas clinically presenting as multifocal lesions in relatively recent diagnosed and untreated AIDS patients. FNA was proven to be effective, and less invasive, in the diagnosis of PHL.

1619 Immunohistochemistry for *Aspergillus sp.* with a Anti-Aspergillus Polyclonal Antibody: Comparison with In Situ Hybridization

L Glaser, A Ziober, LP Wang, KT Montone. Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Background: Aspergillus infections are often detected on histopathologic review, however, it is well established that accurate species identification, particularly of filamentous fungal infections, can be problematic in formalin-fixed tissue specimens. While fungal culture is often considered the "gold standard" for identification, cultures may be negative, or in turn, may have been performed if a fungal pathogen was not suspected. In situ hybridization (ISH) for fungal rRNA is a means for determining the presence *of Aspergillus sp.* in tissue sections. More recently, commercially available antibodies targeting Aspergillus antigens have been developed. In this study a series of tissue specimens culture positive for *Aspergillus sp.* were studied by IHC and compared to a previously established ISH method for *Aspergillus sp.*

Design: Twenty-five formalin-fixed, paraffin embedded tissue specimens from non-invasive and invasive *Aspergillus sp.* culture positive cases were studied. These included specimens from a variety of tissue sites including lung, skin, sinonasal tract, gastrointestinal tract and, brain. Immunohistochemistry (IHC) was performed using a polyclonal rabbit anti-aspergillus antibody at a 1:1500 dilution. The immunogen was soluble extract from *A. fumigatus, A. flavus, A. niger* and *A. terreus*. ISH was performed with a previously established method using a locked nucleic acid probe targeting *Aspergillus sp.* 18S rRNA sequences. Slides from both the IHC and the IHC procedures were visualized using light microscopy.

Results: The IHC procedure resulted in a positive reaction in all 25 cases of known *Aspergillus* infection. The majority of organisms stained with the antibody and there was minimal background staining of the host tissue. ISH showed a positive reaction in 22/25 cases. In comparison, the IHC showed staining in more organisms than that seen by ISH which also show more background staining in the host tissue. Interestingly, both ISH and IHC staining for *Aspergillus* was weakest in fungal balls in comparison to invasive fungal infections. IHC and ISH on tissue specimens from other filamentous invasive fungal infections including Zygomycetes and Candida were negative.

Conclusions: Both IHC and ISH for Aspergillus can accurately identify *Aspergillus sp.* in tissue sections; however, IHC shows less background and stains more organisms. The fewer organisms seen by ISH may be explained by limited staining in non-viable organisms, the spatial localization of the fungal rRNA, method sensitivity or unknown factors.

1620 Identification of Anaerobic Bacteria Using MALDI-TOF MS Bruker Biotyper System

Y-MS Hsu, C-AD Burnham. Washington University School of Medicine, Saint Louis, MO.

Background: Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a method for identifying clinically relevant microorganisms. To date, data on the performance of MALDI-TOF MS for identification of anaerobic organisms is scarce. The objective of this study was to evaluate the performance of MALDI-TOF MS for identification of anaerobic bacteria using the direct spotting (DS) method, with and without a direct formic acid (FA) overlay.

Design: A total of 102 anaerobic bacteria, including 87 clinical isolates and 15 stock organisms, were tested using the Bruker Microflex LT with Biotyper 3.0 software. All isolates were tested in quadruplicate; in duplicate by DS and in duplicate using DS with a 1 uL FA overlay (DS+FA). The MALDI-TOF MS identification was compared to the phenotypic identification from the clinical laboratory. Full length 16S rDNA gene sequencing was used for discrepant analysis, and taken as the gold standard. A full FA extraction was performed on isolates with a score of <2.000.

Results: For analysis, the isolates were divided into 3 groups: Gram-negative bacilli (GNB), Gram-positive bacilli (GPB), and Gram-positive cocci (GPC). For all groups, the FA overlay (DS+FA) improved the average score and reduced the frequency of unidentified isolates.

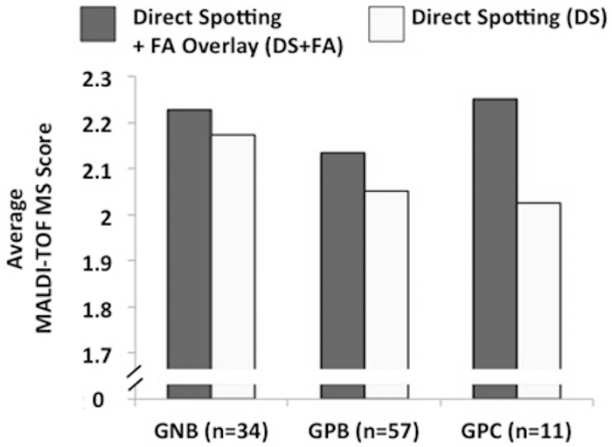

Figure 1. The overall performance of MALDI-TOF MS, using direct spotting (DS) or DS with formic acid overlay (DS+FA) methods, in identifying different morphologic groups of anaerobes.

Overall, 91%(DS)/99%(DS+FA) of isolates were correctly identified to Genus level (score of ≥1.700), and 72%(DS)/86%(DS+FA) of isolates were correctly identified to species level (score of ≥2.000), without misidentifications by either method. For 8 of the 102 isolates (8%), a score of <2.000 was obtained by both DS and DS+FA methods using both the automatic or manual mode on MALDI-TOF MS. A complete FA extraction was performed on these 8 isolates, which yielded identification in only one case (a *Gemella* spp.).

Conclusions: MALDI-TOF MS with Biotyper 3.0 can accurately and rapidly identify clinically relevant anaerobic bacteria. DS with a FA overlay will enhance laboratory workflow by reducing the number of full FA extractions required. MALDI-TOF MS will improve turn-around-time for identification of clinically relevant anaerobes for a very low consumable cost.

1621 Histopathologic Findings of Hepatitis C Virus (HCV) Genotypes 2, 3, and 4 in Liver Biopsies

RSP Huang, A Tholpady, Y Bai, S Vitor, Z Bihong. The University of Texas, Health Science Center at Houston, Houston, TX.

Background: There are at least six known genotypes for HCV and more than 50 subtypes. The most common genotype in the US is type 1 followed by types 2, 3, 4, 5, and 6. Liver biopsies for HCV-infected patients with genotype 1 are commonly performed to assess treatment response and guide treatment regimen. Since liver biopsies are not routinely performed for other HCV genotypes, there is a relative dearth of data for these types. Thus, this study was performed to further characterize histopathologic patterns seen in non-1 HCV genotypes.

Design: This was a retrospective review of 93 patients who had HCV genotype testing between 2007-2011 at an academic medical center. Liver biopsies (where available) were evaluated for stage, grade, and steatosis. In evaluating the liver biopsies, the metavir system was used to score grade and stage between 0-4. Steatosis was scored as mild, moderate, or severe; diffuse or non-diffuse; and micro/macrosteatosis. Demographic data and lab values were collected including HCV viral load and liver function tests (ALT, AST, GGT, alkaline phosphatase, and bilirubin).

Results: Of the 93 patients with non-genotype 1 HCV, biopsies were performed on four HCV-2 infected livers, three HCV-3, one HCV-4, two coinfected with types 1 and 3, and one coinfected with type 1 and 4. There were 9 males, 2 female with a median age of 51 and a range of 29 to 66. The grade, stage, and severity of steatosis for each biopsy is provided in Table 1 along with corresponding lab values. Higher viral loads were typically seen in HCV-2 with mild liver transaminase elevation. The grade and stage for liver coinfected with HCV genotypes were overall more severe than other types singly. Steatosis was seen in all HCV-3 livers.

table 1

TYPE	GRADE	STAGE	STEATOSIS	VIRAL LOAD HCV RNA	LOG10 HCV RNA	AST	ALT
2	2	1	none	6870000	6.8	N/A	N/A
2	2	4	moderate macro/ micro steatosis	170000	5.2	113	118
2	3	4	mild microsteatosis	1190000	6.1	48	68
2	1	4	none	5720000	6.8	107	81
3	3	3	diffuse moderate macrosteatosis	269000	5.4	182	97
3	1	4	mild macro steatosis	6700	3.8	43	63
3	2	2	moderate steatosis	5110000	6.7	N/A	N/A
4	2	0	none	830000	5.9	39	28
1+3	3	3	none	306000	5.5	39	59
1+3	2	4	focal severe macro steatosis	100000	8	275	313
1+4	2	3	mild micro/ macro steatosis	777000	5.9	84	77

Conclusions: Previous studies have shown that HCV-3 is highly associated with steatosis, and our findings support this. Further this study provides data suggesting more severe pathologic changes in livers coinfected with two HCV genotypes. The viral load was overall higher in HCV-2 livers.

1622 Toward a Uniform Reporting of Surgical Specimens with a Diagnosis of Fungal Rhinosinusitis

EM Linde, GA Talmon, SH Hinrichs, PC Iwen. University of Nebraska Medical Center, Omaha, NE.

Background: Fungal rhinosinusitis (FRS) represents 5-12% of rhinosinusitis cases and is broadly categorized as invasive (acute [AIFRS], granulomatous, and chronic) and non-invasive (fungus ball [FB] and eosinophilic to include allergic [AFRS]) disease. Each classification has a distinct clinicopathologic picture and recommended therapy. Multiple studies on FRS have concentrated on disease categorization; however, standardization of reporting has not been described. This study evaluates surgical pathology reports of FRS and provides a template for standardized reporting.

Design: A retrospective review of surgical pathology reports from January 2007 through May 2011 was done to identify all FRS cases. These reports were examined for information on disease classification, fungal elements present, presence/absence of tissue invasion, and etiological agent class/genus. Slides for each case were blindly reviewed and culture data was correlated with the tissue findings.

Results: Seventy-one FRS cases were identified with the initial classifications as unclassified (47.9%), fungus ball (19.7%), AIFRS (15.5%), AFRS (9.9%), and other including chronic sinusitis and allergic mucinous sinusitis (7%). The presence/absence of invasion was specified for each diagnosis as listed: AIFRS (100%), fungus ball (64.3%), other (40%), unclassified (29.4%), and AFRS (14.2%). The percentages where a possible etiological agent class/genus was reported for AIFRS, fungus ball, AFRS, and unclassified were 54.5%, 50.0%, 14.2%, and 23.5%, respectively. Of the 67 cases reviewed, 42 (62.3%) were reclassified based on published criteria as fungus ball (46.2%), AFRS (23.9%), AIFRS (22.3%), and unclassified (7.5%). For all cases, only 31% gave a probable etiological agent class/genus with 13.6% of these culture positive that correlated 66.6% of the time with the histology findings. Only 9.9% of the reports included all four reporting features.

Conclusions: According to published criteria the reporting of pertinent tissue findings in FRS should include disease categorization, description of hyphal elements, presence or absence of tissue invasion, and a probable etiological agent. The results of this study showed that most cases did not use this format and were not properly classified. This highlights a need for a standardized reporting method to help in the management of patients with FRS.

1623 A Simplified Protocol for Rapid Sequence-Based Fungal Identification from Culture or Formalin-Fixed, Paraffin Embedded (FFPE) Tissues

DE Nowak, DA Chitale, R Tibbetts. Henry Ford Hospital, Detroit, MI.

Background: Identification of fungi is often a challenge in clinical microbiology, especially in small samples. Routine culture often results in unidentifiable sterile hyphae or no growth and histologic evaluation is hampered by variable tissue morphotypes. We developed a sequence-based assay using available reagents, kits and equipment familiar to any molecular pathology laboratory. The strengths of this approach include: simplicity, definitive results, and species-specific data in less than 12 hours.

Design: Culture fungi were selected from in-house ATCC stocks and sterile hyphae cultured fungi. FFPE specimens for analysis were selected randomly based on tissue source from archival tissues retrieved from pathology files. Prior diagnoses were blinded. DNA was extracted (QIAamp mini kit, Qiagen) and amplified using published primers ITS3 and ITS4, hybridizing to conserved sequences between the 5s and 28s rRNA genes of all fungi. DNA was purified (QIAquick PCR cleanup kit, Qiagen), sequenced (BigDye3.1, ABI) and used to interrogate the NCBI BLAST nucleotide-to-nucleotide database. Species identification was based on best sequence alignment.

Results: DNA was successfully amplified from cultures and FFPE tissues. ATCC isolates of Cryptococcus laurentii, Candida glabrata, Candida parapsilosis, and Candida tropicalis were all verified: 99%, (gb FJ153214.1); 99%, (gb CR380958.2), 100%, (gb HQ263346.1), 98%, (gb JF300164.1) match, respectively. Sterile hyphae from four cases identified: Ajellomyces dermatitidis, 99%, (gb EF592163.1); Pichia kudriavzevii, 98%, (gb HM771638.1); Cladosporium cladosporioides, 99%, (gb JN253511.1); and Coprinellus micaceous, 98%, (gb GU227721.1) match, respectively. Trichophyton rubrum, Cladosporium cladosporioides, and Cladosporium tenuissimum were identified in toe nail with 96% (gb FM178326.1); 96% (gb JN253511.1); 89% (gb Y15966.1) match, respectively. In lung, a tissue diagnosed as Cryptococcus sp.was identified as Ajellomyces dermatitidis by sequencing with 99% match (GB EF592163.1). Candida sp. with previous histological diagnosis in lung, skin, and esophagus identified as Candida albicans with 93%, (gb HQ014713.1); 99%, (gb HQ014713.1); 96%, (gb HQ014723.1) match, respectively.

Conclusions: We have established an assay for fungal identification based on DNA sequence that is both faster and more definitive than culture or tissue morphology with a unified workflow for fresh, frozen or FFPE fungi in less than 12 hours.

1624 *Mycobacterium Chimaera* an Unusual Cause of Mitral Valve Endocarditis

M Roessle, Y Achermann, D Zimmermann, B Hasse, V Deggim, M Hoffmann, J Grunenfelder, M Hombach. University Hospital Zurich, Zurich, Switzerland; University of Zurich, Zurich, Switzerland; Cantonal Hospital St. Gallen, St. Gallen, Switzerland.

Background: Mitral valve endocarditis (MVE) is mostly caused by bacterial or fungal infections. Only in rare cases non-tuberculous mycobacteria (NTM) were detected as causative agents. Here we present the first case of MVE caused by *Mycobacterium chimaera*.

Design: A 58 years old man with diagnoses of systemic sarcoidosis, therefore receiving steroids, and monoclonal gammopathy of undetermined significance underwent mitral and aortic valve replacement due to severe valve insufficiency. Histological and molecular analyses of mitral valve tissue were performed.

Results: Histologically, there is a partly necrotizing, acute and chronic inflammation of fibrous valve tissue with numerous swollen macrophages containing many PAS- and acid-fast-positive bacteria. Molecularbiological analysis of the FFPE tissue showed mycobacterial DNA of the *M. avium complex*. Subsequent microbiological analysis of the mitral valve annulus, blood cultures, and sputum allowed identification of *M. chimaera* DNA.

Conclusions: We report the first case, in which *M. chimaera* was identified as causative agent of endocarditis in a patient with hitherto unknown disseminated NTM-infection. As - like in our case - NTM-associated endocarditis shows morphological characteristics of NTM-infection of other organs, it is recommended to perform special stains like PAS and Ziehl-Neelsen to highlight the acid-fast bacteria. Subsequent molecular- and microbiological analyses usually allow identification of the mycobacterium species.

1625 Pathologic Studies of Cases with Fungal Soft Tissue Infections after a Tornado – Joplin, Missouri, 2011

W-J Shieh, C Drew, S Lockhart, CA Taylor, L Liu, D Blau, C Paddock, L Gade, RN Fanfair, G Turabelidze, BJ Park, ME Brandt, SR Zaki. Centers for Disease Control and Prevention (CDC), Atlanta, GA; Missouri Department of Health and Senior Services, Jefferson City, MO.

Background: On May 22, 2011, an EF-5 tornado struck Joplin, Missouri, resulting in approximately 1,000 injuries and 159 deaths. A local physician initially identified two hospitalized patients with tornado injuries and necrotizing fungal infections in soft tissues. The Missouri Department of Health and Senior Services initiated active surveillance for such infections with epidemiologic and laboratory assistance from the Centers for Disease Control and Prevention (CDC).

Design: Tissue specimens from surgical debridement of 10 suspect cases were submitted to Infectious Diseases Pathology Branch (IDPB) at CDC for pathologic evaluation and testing. Histopathologic examination, special stains, and immunohistochemical (IHC) assays were performed at IDPB. DNA was extracted from paraffin tissue blocks and submitted to Mycotic Diseases Branch at CDC for molecular testing.

Results: Microscopic examination showed necrotizing inflammation in the soft tissues of all 10 cases. GMS and PAS stains demonstrated scattered fungal hyphae with bizarre shape, haphazard branching, and pauciseptation in 9 cases. Involvement of vascular wall with necrosis was observed in 4 cases. The above 9 cases were all positive for mucormycete fungi by IHC assay and 6 of them were further confirmed as the mucormycete *Apophysomyces trapeziformis* by PCR assay and by culture of the tissue. There were 3 IHC-positive cases with no fungal DNA amplified from the paraffin-embedded tissues. The single case negative for mucormycetes by IHC showed a mixture of small yeasts and septated fungal hyphae with right-angled branching. This case was positive for both *Candida* species and *Aspergillus* species by IHC, and PCR was positive for *Fusarium* species.

Conclusions: Many fungi can be present in traumatic wounds associated with natural disasters. Histologic evaluation, special stains, and IHC can demonstrate these fungi in the surgical debridement samples with corresponding tissue reactions. Therefore, pathologic studies in conjunction with molecular testing are essential to confirm the pathogenic role of these fungi in the wound infections. In this study, the mucormycete *Apophysomyces trapeziformis* was identified as the most frequent fungal pathogen in patients with tornado-associated soft tissue infections in Joplin, Missouri, 2011.

1626 Human Herpesvirus Type 8 in Patients with Child-Pugh Class A to C Cirrhosis

C-C Su, M-N Lin, K-C Tseng. Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan; Tzu Chi University, Hualien, Taiwan.

Background: Human herpesvirus type 8 (HHV-8) DNA has been found consistently in all types of Kaposi's sarcomas (KSs). KS can develop in human immunodeficiency virus (HIV) non-infected patients with variable immunologic abnormalities. Previous studies have been found that patients with Child–Pugh class B/C cirrhosis have a significantly greater seropositive rate for HHV-8 antibodies compared with healthy controls. Those studies did not specifically exclude diabetic patients. It is well known that patients with diabetes have an increased risk of infection with opportunistic diseases

and that the risk of classic KS is four-fold higher in patients with diabetes. There have been no comprehensive studies regarding patients with pure cirrhosis from Child–Pugh class A to C until to now.
Design: Cell counts and HHV-8 antibody and DNA were detected in blood samples from 108 cirrhotic patients without diabetes and 108 age- and sex-matched healthy controls.
Results: Mean lymphocyte, monocyte, and platelet counts were significantly lower, higher, and lower in Child-Pugh class C than class A cirrhotics (each $P < 0.02$), respectively. Monocyte counts were significantly greater in male and class B cirrhotics than female and class A or C cirrhotics (each $P < 0.05$), respectively. Hepatitis C virus (HCV)-infected cirrhotics had significantly lower lymphocyte and platelet counts than alcoholic patients and hepatitis B virus (HBV) infected or alcoholic patients (each $P < 0.05$), respectively. Seropositive rates and titers for HHV-8 antibodies were significantly greater in patients, particularly male and class B or C or HCV-infected cirrhotics, than the controls (each $P < 0.0001$). Seropositive female patients were significantly older than seropositive male patients ($P = 0.0130$), respectively. The rates and titers were also significantly greater in class B or C than class A cirrhotics (each $P < 0.05$). One HCV-infected male patient was positive for HHV-8 DNA (98 copies/mL).
Conclusions: Lymphopenia, monocytosis, and thrombocytopenia and seropositive rates and titers for HHV-8 antibodies were significantly greater in advanced versus mild cirrhotics. Monocytes increased, boosted, and then significantly decreased with Child-Pugh classes.

1627 Invasive Candidiasis Associated with Jejunal Ulceration and Perforation: An Under-Recognized Entity? Report of Three Cases

JNM Tan, MJ O'Brien, NS Miller, H Xu. Boston University Medical Center, Boston, MA.
Background: Candida species, a known colonizer of mucus membranes also has the ability to colonize and invade host cells and tissues to cause infection. The most susceptible patient groups include the immunocompromised, neutropenic patients, low birth weight infants and neonates with severe gastrointestinal diseases. More recent reports suggest association with certain diets and medications such as H2 blockers. A recent case of small bowel ulceration and perforation with invasive candidiasis prompted us to review previous cases of small bowel perforation at our institution.
Design: A restrospective review of all surgical cases of small bowel perforations received at Boston Medical Center from January 1, 2001 to September 30, 2011 was performed. Total of 44 cases were identified; the majority were secondary to trauma (43%), idiopathic causes (30%) and malignancy (9%). Less frequent causes were ischemic enteritis (5%), diverticulosis (5%) and foreign body (2%). Three cases of small bowel perforations (7%) were found associated with invasive *Candida* infection. Histological sections of the involved bowel segments, special stains (PAS and GMS), and microbiology culture results were reviewed.
Results: Histopathology of the segments of resected small bowel in all 3 patients showed invasive *Candida* enteritis with ulceration and perforation. All underwent exploratory laparotomy where perforation of the jejunum was found. The specific organisms subsequently grown in the abdominal drainage/peritoneal fluid sent for culture were *Candida albicans, Candida tropicalis* and *Candida glabrata* respectively, with all three organisms present in one case. No other etiology for the perforation was identified and none were immunosuppressed.

Table 1. Demographic and Clinical Presentation

Case	Age/ Gender	Associated Condition	Presentation	Site of Perforation
1	52/M	Gastrinoma	Acute abdominal pain	Jejunum
2	64/M	s/p Splenectomy 2° trauma	Small bowel obstruction	Jejunum
3	52/M	Liver Failure, Hepatitis C cirrhosis, Portal Vein Thrombosis, Anemia	GI bleed	Jejunum

M = male, s/p = status post, 2° = secondary, GI = gastrointestinal
Conclusions: Invasive Candida infection may be a primary cause of ulceration and perforation of the jejunum. Few cases have been reported in the literature but it is likely to be an under-recognized as well as a rare entity.

1628 H. pylori Infection Is Associated with DNA Damage of Lgr5-Positive Epithelial Stem Cells in Human Stomach

T Uehara, Y Yuan, M Feldman, A Ziober, H Ota, A Sepulveda. University of Pennsylvania, Philadelphia, PA; Shinshu University, Matsumoto, Japan.
Background: The G-protein coupled receptor Lgr5 is expressed in gastric antral epithelial stem cells and in cancer tissues. Mutagenesis of the epithelial stem cell genome has been proposed to underlie gastric cancer (GC) development. 8-hydroxydeoxyguanosine (8OHdG) is a DNA modification induced by reactive oxygen species that can be used to determine levels of DNA injury at the individual cell level. Studies have shown that H. pylori and associated inflammation are main GC risk factors, and this may be related to increased mutagenesis of the gastric epithelium. The aim of our study was to determine whether Lgr5-positive gastric epithelial stem cells are susceptible to mutagenesis associated with H. pylori infection.
Design: Lgr5 and 8OHdG expression was characterized in non-neoplastic gastric antral mucosa of gastrectomy specimens from 24 patients (16 with GC: 9 Hp positive (Hp+), 7 Hp negative (Hp-); and 8 without GC, all Hp-). To determine the extent of mutagenesis in Lgr5 positive epithelial cells (Lgr5+), expression of nuclear 8OHdG, a marker of DNA mutagenesis, was determined in co-stains with Lgr5 by immunofluorescence. Primary Lgr5 rabbit polyclonal and 8OHdG monoclonal mouse antibodies, followed by fluorescence labeled antibodies were used. Quantification of 8OHdG was done by spectral image analysis using a Nuance Trio microscope and CRI imaging software. In each case, at least 10 Lgr5+ and 10 Lgr5- cells from contiguous glandular cells were scanned.
Results: Overall the 8OHdG fluorescence maximum intensity (FMI) in Lgr5+ (mean 313.1, SD 143.1) was significantly higher than in Lgr5- cells (mean 286.5, SD 134.9) P=.03. In the group of GC cases in which both Hp+ and Hp- cases were available, the 8OHdG FMI (mean 263.6, SD 126.9) in Lgr5+ was significantly higher than in Lgr5- cells (mean 215.0, SD 106.5), P=.012 in Hp+ cases but not in Hp- cases (8OHdG FMI mean 342.8, SD 148.0 in Lgr5+ and mean 329.4, SD 134.8, in Lgr5- cells, P=.41).
Conclusions: These data suggest that DNA damage occurs in Lgr5+ gastric epithelial stem cells and that these cells may be more susceptible to oxidative stress than other gastric glandular cells. This is supported by the finding that 8OHdG accumulation was higher in Lgr5+ (stem) cells as compared to Lgr5- cells in patients with active H. pylori infection but not in those without H. pylori infection. These data support a role for H. pylori infection in mutagenesis of gastric stem cells which may underlie H. pylori associated gastric carcinogenesis.

1629 Clinical Significance of Isolated Cytomegalovirus Infected Intestinal Cells

L Wang, J Dennis, Z Yan, C Doern, JY Park. UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.
Background: Cytomegalovirus (CMV) infection of the intestinal tract is associated with high mortality in immunosuppressed patients. However, few studies have correlated peripheral whole blood CMV viral loads with histopathologic findings. Furthermore, there have been few studies determining the clinical significance of isolated CMV infected cells identified by hematoxylin & eosin staining and/or immunohistochemistry (IHC).
Design: We searched all non-consultation intestinal biopsies from 2006 to 2011 using our laboratory information system and further analyzed the cases with the diagnosis of CMV infected cells. We then selected as a control group thirty-one consecutive intestinal biopsy cases that had a negative CMV immunohistochemistry. The electronic medical record was reviewed for each case to determine peripheral blood CMV viral load detection by quantitative PCR, clinicopathologic features at time of diagnosis and clinical outcomes after discharge.
Results: Thirty-one patients with CMV positive intestinal biopsies confirmed by IHC were identified from 52% male and 48% female patients. The clinical setting of the 31 patients with CMV positive biopsies were solid organ transplantation (n=6), bone marrow transplantation (n=6), inflammatory bowel disease (n=6), ischemic colitis (n=6), chemotherapy for solid tumors (n=4), end-stage renal disease (n=3), and infection by Human Immunodeficiency Virus (n=1). CMV viral inclusions were identified by H&E stain in 26% (n=8) of cases, the remaining cases were identified by CMV IHC alone. CMV blood viral load was only positive in 16% (n=5) of cases. None of the negative control cases had positive blood viral loads. Seven cases that had only a single positive CMV infected cell; these cases had the following outcomes: worsening clinical symptoms that responded to antiviral therapy (n=3); did well without treatment (n=3); died after discharge to hospice without treatment (n=1).
Conclusions: CMV infection of the intestines is clinically significant, but will not always present with classic viral cytopathic changes. CMV IHC should be considered in any case where there is clinical suspicion. The identification of a single CMV infected cell by IHC should be regarded as clinically significant. Peripheral blood viral load has poor sensitivity in detecting CMV intestinal infection. However, future studies will investigate whether a positive viral load in patients with intestinal symptoms is predictive of intestinal CMV infection.

Informatics

1630 MeSH Term Trends in Pathology, a PubMED Survey of Articles in Pathology Journals

S Balci, B Dursun, T Ozyer. Yildirim Beyazit University Ataturk Hospital, Ankara, Turkey; TUBITAK, Ankara, Turkey; TOBB University, Ankara, Turkey.
Background: Medical Subject Headings are tagged for each journal article in NCBI database. We wanted to use MeSH terms to observe the alterations in research topics of pathology.
Design: 76 journals whose subject category were pathology in ISI Web of Knowledge Journal Citation Reports 2010 were included. ISSN numbers of these journals were used to download 223787 articles from PubMED in MEDLINE format. 2011 MeSH tree, journal and article properties in MEDLINE format were imported into Oracle database. Number of articles for each term was determined mimicking PubMED's explode function. To correct the numbers in regard to the increase in the publication numbers, term number was divided by article number to define a percentage. These percentages were used to compare the ratio between the last two 5-year-periods and named percent ratio.
Results: Although pathology journal articles constituted 1% of more than 21 million articles in PubMED, they covered 72.6% of total MeSH terms (26140). Last two 5-year were used to determine the recent changes in research fields; where >110% ratio was accepted as increase, <90% was decreased, others as relatively stable. In example, articles tagged with Molecular Diagnostic Techniques, Forensic Pathology, Surgical Pathology, Digestive System Neoplasms and Urogenital Neoplasms were increased whereas Immunohistochemistry and Telepathology were decreased.