expression is seen in approximately 30% of DLBCL, which is unlikely caused by *ERG* rearrangement as seen in prostate carcinoma. Targeted therapy against ERG might be beneficial for patients with FL and ERG+ DLBCL.

1553 Cytogenetic and Molecular Features of 59 Cases of Bone Marrow Failure Syndrome

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Background: Bone marrow failure is multilineage cytopenias and hypoplasia and includes a heterogeneous group of disorders, most commonly aplastic anemia (AA), hypoplastic myelodysplastic syndrome (MDS), paroxysmal nocturnal hemoglobinuria (PNH), and T-cell large granular lymphocytosis (T-LGL). There are overlapping clinical and morphological features among these entities, and often times definitive diagnosis cannot be rendered at initial presentation. This study is to examine the cytogenetic and molecular features of a cohort of bone marrow failure cases, and summarize their cytogenetic and molecular features.

Design: We retrieved 59 cases with cytopenias and hypoplastic bone marrow at Moffitt Cancer Center, on which targeted next-generation sequencing (54 myeloid neoplasm related genes) was performed. Peripheral blood complete blood counts, bone marrow morphological findings, flow cytometric analyses for LGL and PNH, cytogenetics and molecular data were extracted from electronic medical records and summarized.

Results: The 59 patients showed peripheral blood cytopenias and bone marrow aplasia or marked hypocellularity. There were 36 cases diagnosed with AA with no morphologic evidence of dysplasia or increased blasts in the bone marrow, 21 cases diagnosed with hypoplastic MDS based on morphological and cytogenetic criteria, and 2 cases diagnosed with T-LGL. Among the 36 cases of the AA cases, hypoplastic MDS remained in the differential diagnosis at the initial diagnosis, and T-LGL population was identified in 4 of 18 cases (22.2%) and 3 of 11 cases with hypoplastic MDS (27.3%); PNH clones were identified in 9 of 22 cases with AA (40.9%) and 3 of 11 cases with hypoplastic MDS (27.3%). Clonal cytogenetic abnormalities were found in 8 of 32 cases with AA (25%) and 12 of 20 cases with hypoplastic MDS (60.0%) and none of T-LGL cases. Eighteen of 36 cases of AA (50%) showed one or more gene mutations. In contrast, 16 of 21 cases of hypoplastic MDS (76.2%) had one or more gene mutations, and 0 of 2 T-LGL cases had mutations. Three cases diagnosed with AA eventually evolved to overt hypoplastic MDS, and all of the 3 cases had two or more gene mutations.

Conclusions: Clinicopathological, cytogenetic and molecular features are summarized in this cohort of bone marrow failure cases. These features may help to make differential diagnosis and identify the cases with more progression potential. Clinical outcomes with different treatment and larger scale studies are needed to better characterize and define the two different entities.

1554 Validation of Mutant Calreticulin Immunohistochemistry (IHC) in Myeloproliferative Neoplasms (MPN)

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Background: Somatic mutations in *Calreticulin* (*CALR*) are the second most common driver mutation in primary myelofibrosis and essential thrombocythemia. They are essentially mutually exclusive of *JAK2*V617F and *MPL* mutations. All pathogenic *CALR* mutations result in a 1bp frameshift (FS) and mutant protein with a novel C-terminus. We report the validation of CAL2, a commercially available monoclonal antibody against the mutant CALR C-terminal neo-epitope, for clinical diagnostic IHC use and its correlation with molecular status.

Design: 54 bone marrow (BM) cases were retrieved from tissue archives, including 48 molecularly-defined MPNs with *CALR* FS mutations (24/48), *JAK2*V617F (12/48), MPL mutations (9/48), and triple-negative (TN) for *CALR*/*JAK2*/*MPL* (3/48), respectively. 3 BM cases with *CALR* in-frame (IF) deletions, 3 normal BMs and a tissue microarray (TMA, 73 samples, 30 normal tissues, 15 solid tumors, and B-cell lymphoma) were also used. Mutant CALR antibody (CAL2, Dianova, Germany) was titered at 1/50 following manufacturer's instructions. Slides were stained on Ventana Benchmark XT (92 mins CC1 epitope retrieval, 32 mins antibody incubation, and 8 mins OptiView detection plus amplification).

Results: Among 22/24 MPN cases harboring 9 various sizes of *CALR* FS mutations, strong CAL2 cytoplasmic staining (intensity 2-3/3) was readily recognizable in the megakaryocytes (MK). 2/24 cases were equivocal for CAL2 IHC as both were fibrotic with rare MK present. 3/3 cases with *CALR* IF deletions were negative by CAL2 IHC. Two had no features of MPN and the last was a *CALR* FS MPN 3-month post stem cell transplant (Tx). Although suspicious morphology was seen, the negative CAL2 IHC supported a molecular remission along with the absence of the pre-Tx FS mutation, 100% donor chimerism, subsequent morphologic resolution, and post-Tx IF deletion (from donor). No CAL2 staining was seen in the 12 *JAK2*V617F, 9 *MPL* and 3 TN MPN cases, or in the 3 normal BMS and 73 TMA samples. CAL2 IHC results were congruent with the molecular status in both B5 and AZF-fixed BM biopsies. Concordant staining was seen in 6 paired BM core biopsies and clot sections.

Conclusions: CAL2 IHC showed 100% specificity for MPNs harboring *CALR* FS mutations, differentiating them from cases with non-pathogenic *CALR* IF variants and MPNs with other driver mutations. The sensitivity of CAL2 IHC was 92%, being limited in cases of markedly fibrotic BMs with minimal MKs. We successfully validated CAL2 IHC for clinical use on both BM core biopsies and clot sections.

1555 Therapy-Related Myeloid Neoplasm Has a Higher Subclonal Mutation Burden Than De Novo AML

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Background: Therapy-related myeloid neoplasms (t-MN) are complications of cytotoxic therapy. They have a higher incidence of *TP53* mutation relative to de novo acute myeloid leukemia (AML). The diagnosis of t-MN is inherently uncertain, either because a treatment history may be missing or because prior treatment may not be causal. Hypothesizing that a genetically-based classification may be more accurate, we employed a 643 gene next generation sequencing panel to study subclonal mutations in t-MN and AML, and also compared their mutational landscape and clinical features.

Design: We collected sequencing data from the diagnostic marrow or blood samples from 20 consecutive patients with t-MN and 23 consecutive patients with AML. Clinical, hematological, and cytogenetic data were tabulated. The bioinformatics pipeline screens noise on a per-base level before variant calling. The main clonal mutation was assumed to be the mutation with the highest variant allele frequency (VAF). Subclonal mutation burden was defined as the number of mutations 1) with VAF $\leq$50% of the main clonal VAF, 2) with VAF $\leq$25% and 3) in which the low VAF cannot be explained by cytogenetic changes.

Results: Mutations of *TP53* are enriched in t-MN cases as compared with AML: 35% (7/20) vs 13% (3/23). Interestingly, $\geq$2 *TP53* mutations are specific for t-MN in this cohort: 25% (5/20) vs 0% (0/23). t-MN in young patients (<40) are less likely to carry *TP53* mutation: 14.2% (1/7) vs 46.2% (6/13). Subclonal mutations are found in all cases of t-MN (range of mutations per patient: 1-30), and in 91% of AML (range: 0-10). On average, t-MN has 8.5±7.6 subclonal mutations, while AML has 3.5±2.5 mutations (P=0.0047). t-MN with $\geq$5 subclonal mutations is positively associated with *TP53* mutation (50% (6/12) vs 12.5% (1/8)). Notably, there are two t-MN carrying *inv(16)*/t(16;16), both of which have only 1 subclonal mutation.

Conclusions: t-MN harbor more subclonal mutations than AML, though t-MN are heterogeneous in terms of subclonal mutation burden. t-MN with few subclonal mutations, including those genetically defined entities like inv(16), are less frequently associated with *TP53* mutation, raising the question whether they are clinically distinct and not caused by prior therapy. The findings lay the groundwork for a larger study to assess the clinical significance of subclonal mutation structure.

1556 Secondary t(9;22)(q34;q11.2)/*BCR-ABL1* Rearrangement Is a Fatal Event in Myeloid/Lymphoid Neoplasms

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Background: t(9;22)(q34;q11.2)/*BCR-ABL1* is a defining event for chronic myeloid leukemia (CML). It is also commonly observed in *de novo* B-lymphoblastic leukemia (B-ALL), and rarely in *de novo* acute leukemia of ambiguous lineage, T-ALL, or acute myeloid leukemia (AML). t(9;22)(q34;q11.2)/*BCR-ABL1* acquired as a secondary event during the disease course is rare. Here we report a series of such myeloid/lymphoid neoplasms.

Design: Cases with t(9;22)(q34;q11.2)/*BCR-ABL1* diagnosed from 1998 to 2016 were reviewed. Patients with the following diseases with t(9;22)(q34;q11.2)/*BCR-ABL1* were excluded: chronic myeloid leukemia, *de novo* B- or T-ALL, *de novo* acute leukemia of ambiguous lineage, and *de novo* AML.

Results: A total of 14 patients with myeloid/lymphoid neoplasms with secondary t(9;22)(q34;q11.2)/*BCR-ABL1* were identified. There were 6 men and 8 women with a median age of 58 years (range, 18-78 years) at initial diagnosis. At initial diagnosis, 7 patients had AML, 6 had myelodysplastic syndrome (MDS), and 1 had T-ALL. Of 7 patients with initial diagnosis of AML, 6 acquired t(9;22) at relapse of AML, and 1 acquired t(9;22) during treatment of refractory disease. Of 6 patients with initial diagnosis of MDS, 5 developed secondary AML (sAML) and acquired t(9;22) when sAML developed (n=1), progressed (n=2) or relapsed (n=2), and 1 developed MDS with excess blasts. In the patient with T-ALL, t(9;22) was acquired during disease progression after multiple relapses. When t(9;22) was acquired, none of 14 patients had a picture of CML in the peripheral blood. Of 9 patients with *BCR-ABL1* transcript type available, 8 had e1a2 and 1 had b2a2. For 11 patients with treatment information available, 10 received chemotherapy and 5 received tyrosine kinase inhibitors. Follow up information was available for 12 patients: all died within 9.3 months with a median survival of 1.8 months.

Conclusions: Secondary *BCR-ABL1* is a rare event in myeloid and lymphoid neoplasms. The vast majority of patients acquire t(9;22) at relapse of *de novo* or secondary acute leukemia. When t(9;22) is acquired, no morphologic features of CML are seen. Also different from myeloid blast phase of CML, the transcript type in acute leukemia with secondary *BCR-ABL1* is overwhelmingly e1a2, which encodes p190 protein. The prognosis of patients with secondary t(9;22) is extremely poor.

Infectious Disease Pathology

1557 Intestinal Spirochetosis Histologic and Immunohistochemical Patterns: A Case Series

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Background: Intestinal spirochetosis is a well described infection associated with *Brachyspira (Br)* spp. Diagnosis is made by histology as growth is not possible with routine culture. The spirochetes are visualized as a basophilic brush border on the luminal surface of colonic mucosa and are not usually associated with a brisk inflammatory

infiltrate. However, some cases have been reported with a lymphoplasmacytic infiltrate mimicking *Treponema pallidum* (*Tp*) spirochetosis. This is further confounded by previously described cross-reactivity for *Br* spp. with *Tp* immunostain (IHC).
Design: All H&E slides of cases with positive *Tp* IHC were reviewed from 1997 to August 2016. Grade and location of inflammation and pattern of spirochetes were recorded. Syphilis serology and patient (pt) follow-up were obtained by chart review.
Results: Of the 6 cases, all pts were men (mean 36 years) who presented with hematochezia. Colonoscopy showed ulcers in the rectum (83%) and sigmoid colon (16%). Mild to severe lymphoplasmacytic inflammation involved the lamina propria (LP) and submucosa (SM). Spirochetes detected by *Tp* IHC involved the crypts and LP.
Table 1

Case	Lymphoplasmacytic Inflammation		Spirochetes by Tp IHC				
	Grade	Location	Burden	Crypt Surface	Crypt Epithelium	Crypt Basal Layer	LP
1	Severe	LP/SM	Moderate	-	-	-	+
2	Mild	LP	Mild	-	-	-	+
3	Moderate	LP	Moderate	-	+	+	+
4	Severe	LP/SM	Severe	-	+	+	+
5	Moderate	LP	Moderate	+	+	-	-
6	Severe	LP/SM	Moderate	+	+	-	-

In two cases, spirochetes were limited to the crypt surface and epithelium. These two pts had non-reactive rapid plasma regain (RPR) and one had a non-reactive fluorescent treponemal antibody absorption test (FTA). Of the pts with LP spirochetes, 50% had a reactive RPR and all had reactive FTA. Clinically, pts with crypt spirochetes were diagnosed with intestinal spirochetosis and pts with LP spirochetes were diagnosed with rectal syphilis.
Conclusions: These cases show the challenges of using *Tp* IHC without considering the staining pattern of the spirochetes. Both cases of intestinal spirochetosis showed cross-reactivity with *Tp* IHC, and spirochetes limited to the crypt surfaces and epithelium. Given the lymphoplasmacytic infiltrate, an incorrect diagnosis of rectal syphilis could be made. Clinical correlation with serology proved that neither of these patients had syphilis. Overall, this case series illustrates the need for consideration of intestinal spirochetosis when spirochetes do not involve the LP and serology is non-reactive.

1558 Modified Acid Fast Stains and Molecular Diagnostics for Early Diagnosis of *Legionella micdadei* Infection

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Background: *Legionella micdadei* is an environmental Gram negative rod and a common cause of *Legionella* pneumonia. *Legionella* requires charcoal-containing media with 3-5 days needed for culture-based identification. Many centers only offer *L. pneumophila* serogroup 1 (sg1) urine antigen testing due to culture requirements and turnaround time. Early detection of all *Legionella* species can be accomplished using molecular techniques. In addition, *L. micdadei*, which is weakly acid fast, may be detected on modified acid fast stains (mAF).
Design: To evaluate the utility of mAF stains, results from all mAF stains and *Nocardia, Legionella,* lower respiratory, and acid fast bacilli cultures/stains were reviewed from 1/2011 through 6/2016 in a tertiary care center with a significant immunocompromised population and endemic *L. micdadei*. The ability of histopathological stains (GMS, AFB, Warthin-Starry, and FITE) to detect *L. micdadei* was evaluated using a representative autopsy. Adjacent tissue was submitted to the University of Washington Clinical Microbiology Laboratory, Molecular Diagnosis Section for 16S rDNA *Legionella* genus-specific PCR with speciation determined by amplicon sequencing.
Results: Over a 5.5 year period, 18/1901 (0.95%) of all mAF stains were positive (15/1282 patients). Corresponding cultures grew *L. micdadei* from 3/15 patients, *Nocardia* species from 8/15 (3 *N. farcinica*, 1 *N. nova*, 1 *N. gamkensis*, 2 *N. cyriacigeorgica*, and 1 *Nocardia* species related to *N. nova*), and 4 positive stains that did not grow any mAFB organisms in culture. *L. micdadei* was detected on mAF stains in 3 of 4 patients which grew the organism. The time difference between mAF stain and culture result was between 1-5 days. FITE and Warthin-Starry stained histopathologic sections identified organisms consistent with *L. micdadei.* Tissue submitted for PCR and *s*equencing confirmed the presence of *L. micdadei* DNA.
Conclusions: Early diagnosis of *Legionella* is critical for appropriate antimicrobial therapy. The availability of rapid PCR and sequencing assays, with performance times within 2 days, has the potential to reduce the time to final diagnosis for all *Legionella* species and provide a molecular diagnosis when cultures are negative or not available. In centers without species-specific PCR, mAF stains (including FITE) can provide rapid presumptive identification of *L. micdadei since t*he small Gram negative rods of *L. micdadei* are easily distinguished from the branching Gram positive rods of *Nocardia* species.

1559 Helicobacter Pylori Clarithromycin Resistance Mutations Are Common and Associated with Increased Treatment Failures: San Diego Helicobacter Pylori Antibiotic Resistance (SD HELP) Study

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Background: *Helicobacter pylori* (*H. pylori*) remains one of the world's most common pathogens infecting approximately 50% of the population and leading to complications including gastric carcinoma and lymphoma. It has been established that *H. pylori* antibiotic resistance is increasing globally, but this phenomenon has been less well studied in the United States.
Design: To determine the local rate of *H. pylori* antibiotic resistance, we performed a retrospective analysis of 23S rRNA gene mutations that confer clarithromycin resistance. From 2013 to 2015, 165 patients were identified who met the following inclusion criteria: underwent gastric endoscopic biopsy, had *H. pylori* gastritis with >50 organisms on a single histologic slide section, and had clinical and molecular data available. 23S rRNA domain V was amplified by PCR then sequenced using DNA extracted from 100 micrometer sections of formalin-fixed paraffin-embedded tissue. Clinical data were obtained using the electronic medical record.
Results: Overall, approximately 7% of gastric biopsies were positive for *H. pylori* and had the following demographics: age (mean 55.4 years, range 19–85 years), gender (female 60.6%), and ethnicity (Hispanic 27.9%, Caucasian 24.8%, Asian 18.2%, African American 10.9%, Other 18.2%). Of the 165 *H. pylori* positive cases evaluated, 52 had mutations in the 23S rRNA gene (31.6% of cases). Only two different mutations were seen, A2142>G (n=10, 19.2%) and A2143>G (n=42, 80.8%). Importantly, harboring one of the 23S rRNA mutations was associated with a significantly increased rate of initial treatment failure (OR 4.2, p=0.002).
Conclusions: *H. pylori* infection and antibiotic resistance represent a significant problem globally. These data from a large single institution cohort with robust clinical follow-up data confirm that clarithromycin antibiotic resistance mutations occur in a significant proportion of cases (31.6%) in San Diego. Moreover, these mutations contribute significantly to treatment failure. These findings suggest that empiric first line therapy using clarithromycin may not be appropriate for all patients and that up front antibiotic sensitivity/resistance testing should be performed prior to initiation of antibiotic therapy.

1560 Unsuspected Spirochetosis in Receipts of Bone Marrow Transplant: An Institutional Review of Case Series

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Background: Patients received bone marrow transplantation (BMT) are at high risks for a wide spectrum of infectious etiologies due to the impairment of cell-mediated and humoral immunity, and immunosuppressive regimen. This immuno-compromised state makes these patients prone to infections by microorganisms that are usually dormant in immuno-competent individuals; one such frequently overlooked disease is Gastro-Intestinal (GI) spirochetosis, caused by Brachyspira pilosicoli and Brachyspira aalborgi. Based on our institutional archive, we reviewed the clinicopathological features and diagnostic pitfalls of intestinal spirochetosis in BMT patients.
Design: Gastrointestinal (GI) biopsies of BMT patients dated 2012 to 2016 were retrieved; the pathology reports, slides, disease history and clinical presentation of BMT patients were reviewed; all specimens were GI biopsies sent to pathology laboratory with clinical requests of "ruling out graft versus host disease". Confirmatory Warthin-starry stain was performed when morphological suspicion arises.
Results: Receipts of BMT underwent a sequential suppression of host immunity, and are vulnerable to various opportunistic infections subsequently. Among 210 BMT cases reviewed, majority of patients presented with abdominal discomfort and watery diarrhea. Among the 210 cases, 4 (1.9%) were identified with intestinal spirochetosis, with no unequivocal Graft Versus Host Disease (GVHD) seen. One of the 4 was also diagnosed with GI coinfection by protozoan Toxoplasma gondii, indicative significantly compromised immunity. Administration of Metronidazole swiftly eradicated most of the GI symptoms in these patients; follow-up confirms an absence of spirochetosis.
Conclusions: Brachyspira pilosicoli and Brachyspira aalborgi are frequently unsuspected and underrecognized opportunistic microorganisms in recipients of BMT, due to an increased predilection to infection. Given a GI biopsy from a BMT patient clinically labeled "suspicious GVHD", pathologists need to be aware of subtle histopathological characteristics of spirochetosis and potentially another coinfectious culprit; astute inspection of the microscopic morphology and correlation with clinical parameters such as liver function and laboratory findings are pivotal for optimizing clinical management and patients' outcome.

1561 Detection of Incidental *Helicobacter pylori* Infection with High Rates of Clarithromycin Resistance in Bariatric Surgery Specimens

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Background: *Helicobactor pylori (H. pylori)* is a common pathogen that causes peptic ulcer disease, gastric lymphoma, and gastric carcinoma. Resistance to clarithromycin has emerged as a major cause of treatment failure. Here, we study the rate of incidental significant pathological findings including *H. pylori* infection and assessed for clarithromycin resistance mutations in bariatric surgical specimens.
Design: Sleeve gastrectomy specimens procured during bariatric surgery were identified over an eight year period (2008 to 2016) and pathology reports reviewed for potentially significant pathological findings. In cases with findings, the diagnosis was

confirmed by histological review. In cases of *H. pylori* infection, the corresponding formalin-fixed paraffin-embedded (FFPE) blocks were utilized for extraction of total DNA, then PCR of domain V of the *H. pylori* 23S rRNA gene (and KRAS as a control gene) was performed, followed by sequencing. Clinical data were collected using the electronic medical record.

Results: A total of 222 sleeve gastrectomies were identified, 24 (10.8%) with potentially significant pathology including the following: 16 (7.2%) with *H. pylori* infection demonstrated by routine staining methods, 5 (2.3%) with a non-specific moderate chronic gastritis, 1 (0.5%) with autoimmune gastritis, 1 (0.5%) with lymphocytic gastritis, and 1 (0.5%) with multiple proximal fundic gland polyps concerning for the GAPPS (gastric adenocarcinoma associated with proximal polyposis of the stomach) syndrome. Of the 14 *H.pylori* postive cases that were successfully amplified (2 had DNA degradation), two harbored the 2195 C>T mutation, one harbored the 2182 T>C mutation, and one harbored the 2142 A>G mutation. Thus, 4 of the 14 (28.5%) *H. pylori*-positive specimens contained a mutation associated with clarithromycin resistance. Nine of 14 (64.3%) cases were treated with triple therapy regimens. Eradication was confirmed in 2 cases by stool antigen testing, and there were no post-treatment biopsies performed.

Conclusions: Our study revealed a relatively low rate of potentially significant incidental pathology in sleeve gastrectomy specimens (10.8%) with the majority of these being *H. pylori* infection (7.2%). In addition, there was a high rate (28.5%) of mutations associated with clarithromycin resistance. Our findings suggest that post-treatment confirmation of *H. pylori* eradication may be warranted given the high rate of clarithromycin resistance.

1562 Reproducible Histopathology and Culture Recommendations for Subtle P. Acnes Causing Longterm Joint Pain and Stiffness in Post-Arthroplasty Patients

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Background: *Proprionibacterium acnes* (P. acnes), a fastidious organism rarely identified in native shoulder, may cause post-arthroplasty infection, but may escape detection by usual clinical and laboratory parameters, including absence of tissue neutrophils, negative gram stain, and too-short culture growth-time. Joint infection is usually detected by counting synovial neutrophils. We wanted to correlate the synovial pathologic findings in treatable post-arthroplasty P. acnes infection.

Design: Pathology slides, clinical and microbiology records of patients with known P. acnes or history of continued unexplained post-operative pain and stiffness were reviewed.

Results: Thirty-two post-operative patients (median 74.5 years; 19F;13M; 25R:7L-sided, knee=17, hip=5, wrist=2, shoulder=2, hand=1, foot=1, spine=1, ST=3) were divided into two groups: 1) synovial-culture P. acnes positive by 4-weeks (PAP, n= 17); 2) pathologic-suspicious P. acnes where cultures were not performed, not held long enough, or the patients had received preventive-preoperative antibiotics (PPA, n=15). One PPA patient had opposite uninvolved post-operative knee for pathologic normal control. All patients, except knee control and 1 PPA with only 1 slide-for-review had A) distinctive thick clean fibrin cake without neutrophils or debris and B) zonal marked linear granulation tissue perpendicular to the fibrin. Three cases showed wear-debris; two radiologic evidence of tibial-component-loosening. No patients had positive gram-stain or synovial-fluid. One patient with blood culture positivity for P. acnes developed septic shock. All PAP and PPA patients had similar clinical symptoms of months-to-years of post-operative pain-and-stiffness, without erythema, swelling, warmth, and without serologic evidence for rheumatoid arthritis or Lyme disease. All patients with these pathologic-findings experienced full resolution of symptoms after 6-weeks of IV-antibiotics specific to P. acnes.

Conclusions: P. acnes can be diagnosed at frozen section or permanent as a distinctive thick fibrin cake with perpendicular vasculature, in synovium, even in absence of neutrophils and with negative gram stain, in patients with post-arthroplasty longterm stiffness and pain. Cultures for P. acnes are positive for growth late and best held for four weeks. Reproducible pathologic and culture detection in these patients can optimize treatment and outcome.

1563 Upsurge of Enterovirus D68 Infection in the Lower Hudson Valley, New York, 2016

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Background: In 2014, a nationwide outbreak ofsevere respiratory illness associated with *Enterovirus* D68 was reported in the US. There were no EV-D68 cases during the 2015 enterovirus season per CDC's National Enterovirus Surveillance System (NESS) but in 2016 EV-D68 has been sporadically detected. It is unclear if this upsurge of EV-D68 infections in 2016 is localized or will spread nationwide, and if there are any significant variations in the virus genome or the severity of clinical disease. So, aim of this study was to perform EV-D68-specific rRT-PCR assay and subsequent next-generation sequencing (NGS) to find any significant variation in viral genome from 2014 EV-D68 strain.

Design: Nasopharyngeal (NP) swab specimens from patients with respiratory illness and/or neurologic symptoms were examined for the presence of *Rhinovirus/Enterovirus* (RhV/EV)using the FilmArray™ Respiratory Panel (RVP) assay. Positive specimens were further analyzed with an established EV-D68-specific rRT-PCR assay (J Clin Microbiology, 2015, 53:1915) and next-generation sequencing (NGS) on the Illumina MiSeq. Historical laboratory and clinical data from confirmed cases were also obtained.

Results: From June 1 to September 20, 2016, 331 of 1,161 (28.5%) NP specimens examined by the RVP assay were positive for RhV/EV. EV-D68 was detected by rRT-PCR in 117 of 297 (39.3%) RhV/EV-positive NP specimens. Of 109 laboratory-confirmed cases with clinical data, 64 (59%) were male, age range from 4 weeks to 90 years-old with a median age of 3 years old. The majority of patients presented with respiratory signs and symptoms, including fever (64%) and cough (44%), while neurologic symptoms, suggestive of acute flaccid myelitis, were observed in 1 patient. 22 (19%) patients required pediatric intensive care. Comparative analysis of the nearly complete EV-D68 genomes from 4 patients identified multiple mutations and a subcluster differed from the 2014 endemic strains.

Conclusions: We reported an upsurge of laboratory-confirmed EV-D68 infections in 117 patients of the lower Hudson Valley, New York in 2016. Multiple mutations in the viral genomes of the 2016 EV-D68 strains and variations in the spectrum and severity of clinical diseases were also observed.

* First and second author did the same amout of work.

1564 Evaluation of the Xpert MTB/RIF Assay for the Detection of Tuberculosis in Patients Being Evaluated for Tuberculosis in a Large Public Hospital in the United States

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Background: The Xpert MTB/RIF assay is an automated, nucleic acid amplification test approved for the rapid detection of *Mycobacterium tuberculosis* complex (MTBC) and rifampin-resistance (Rif-R) directly from sputum samples. The performance of the MTB/Rif assay in evaluating patients for pulmonary tuberculosis (TB) has not been extensively studied in high TB prevalence regions within the United States.

Design: Records for all patients who underwent testing with the MTB/Rif assay between November 2015 and March 2016 were retrospectively reviewed. Performance of the MTB/Rif assay was compared to the performance of three AFB smears, using culture as the gold standard.

Results: A total of 512 consecutive adult patients underwent testing with the MTB/Rif assay during the study period, with 1308 AFB sputum smears and 843 MTB/Rif tests performed. These patients underwent evaluation by the emergency department (16%), inpatient service (77%), and outpatient service (7%). 5.47% of patients tested were found to be culture positive for MTBC. Of the 28 patients with positive MTBC cultures, 24 (86%) were positive by the MTB/Rif assay and 18 (64%) patients were smear-positive. Importantly, the MTB/Rif assay was positive in 6 of the 10 cases missed by smear. There were three MTB/Rif-positive, culture-negative cases, all of whom were patients who were undergoing or had recently undergone treatment for TB. The sensitivity, specificity, positive predictive value, and negative predictive value of the MTB/Rif assay were 85.7%, 99.3%, 88.9%, and 99.1%, respectively. Test performance of the MTB/Rif assay was superior to the 3-smear approach, with the area under the curve for MTB/Rif assay and 3-smear testing corresponding to 0.923 and 0.809, respectively ($p = 0.006$). Testing location and type of specimen (raw vs. concentrated) did not appear to influence MTB/Rif assay results.

Conclusions: Although the sensitivity of the MTB/Rif assay is lower than culture, it is superior to the 3-smear approach and led to a more rapid diagnosis of TB in 6 patients who were AFB smear-negative. Due to its high negative predictive value (99.1%), use of the MTB/Rif assay for initial TB evaluation and decision support for discontinuation of airborne precautions is supported by these data, in keeping with recently published guidelines by the National Tuberculosis Controllers Association.

1565 Genomic and Clinical Characterization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Pediatric Patients from a Suburban New York City Children's Hospital

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a major concern worldwide. In the United States, the MRSA strain USA300 with sequence type 8 (ST8) and SCCmecIVa has been predominant in community-acquired MRSA (CA-MRSA) infections. The aim of this study is to assess the population genetics of MRSA in pediatric patients at a suburban New York City children's hospital and the genomic and clinical characterization of MRSA infections.

Design: Clinically relevant MRSA isolates from all pediatric patients with age of 7 or younger and representative MRSA isolates from adults and pediatric patients who were older than 8-year-old from 2014 were included in this study. Genome sequences from all MRSA isolates were achieved using the Illunima MiSeq or HighSeq next-generation sequencing (NGS) systems. Routine microbiology and clinical data were also obtained from patient medical records.

Results: A total of 145 MRSA isolates were included in this analysis. Two major clones of MRSA (ST8 and ST105) were identified among these clinical isolates by whole-genome sequencing. The CA-MRSA clone ST8 was seen in 63 of 82 (76.8 %) isolates from pediatric patients but only in 21 of 63 (33.3 %) isolates from adult patients ($p < 0.001$). By contrast, ST105 clone was seen only in 6 of 82 (7.3 %) isolates from pediatrics but in 30 of 63 (47.6 %) of adult patients ($p < 0.01$). The major clinical syndromes associated with ST8 CA-MRSA in pediatric patients were skin and soft tissue infections (n=58) and invasive infections (sepsis, epidural abscesses, and necrotizing pneumonia) (n=5).

Conclusions: NGS analysis revealed that two major clones of MRSA, ST8 and ST105, were circulating among our pediatric and adult patient populations, respectively. The CA-MRSA ST8 (USA300) was the predominant clone in pediatric patients and was most common in causing skin and soft tissue infections, while ST105 was most frequently found in adult patients. Our study demonstrates the use of NGS for understanding and continuous monitoring of the MRSA clonality in a local community and hospital and its impact on infection control practice.

1566 Acid Fastness of *Histoplasma* in Surgical Pathology Practice: Can Ziehl Neelsen Stain Be an Additional Histochemical Stain for Fungi?

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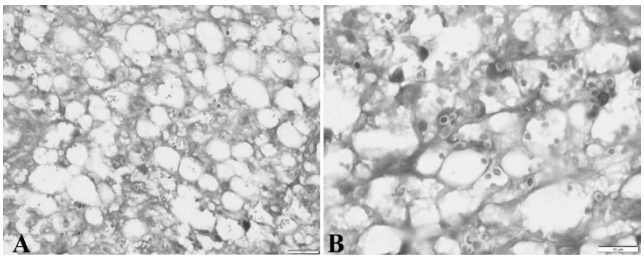
Background: Histoplasmosis (HP) is diagnosed by seeing intracellular organism in biopsy and/or culture. Periodic acid Schiff-diastase (PAS-D) and silver methenamine (SM) are confirmatory stains for HP. Acid fast property of *Histoplasma* has been identified decades ago but it has never been practiced in current surgical pathology. The awareness of acid fast property of *Histoplasma*, due to mycolic acid in the wall, is important in distinguishing it from other infective organisms. We have aimed to examine acid fastness in our previously diagnosed cases of *Histoplasma* by Ziehl Neelsen (ZN) stain and correlate with other known fungal stains.

Design: From 2010 to 2016, a total of 66 cases of HP were diagnosed based on histology and PCR/culture/ serology. Paraffin blocks were available in 43 cases. Deeper levels of each case were stained for ZN, PAS-D, and SM stains. Morphology of *Histoplasma* was reviewed along with fungal stains. Acid fastness of fungus was searched in ZN stain in all the cases.

Results: Patients (39 men, 4 women) had an age range of 11-69 years. Culture/serology was positive in 8 cases. Immune status was known in 31 cases, of which 11 were immunocompromised. Four cases showed entirely necrotic tissue. The most common site was skin followed by adrenal and respiratory tract. Of 43 cases, 20 (46.5%) stained positive with ZN stain. In viable cases a significant proportion of organisms were ZN stain positive.



Necrotic cases showed rare AFB positive yeasts in comparison to viable cases. In comparison to PAS-D and SM stains AFB positive yeasts were low in burden.

Conclusions: This is one of the largest series of HP from India and also the largest series worldwide where acid fastness of *Histoplasma* is evaluated. A simple ZN stain may be included in routine diagnostic armamentarium of fungal infections in surgical pathology. Although every organism is not acid fast within a case, overall approximately half of cases of HP are positive on ZN stain. The surgical pathologist should be made aware of this property of *Histoplasma*, particularly when there is a diagnostic confusion with other infective organisms.

1567 *Toxoplasma gondii* Pru*ΔKU80* and Its Parental Strain Pru: Modulation of Brain Cyst Formation During Chronic Toxoplasmosis from an Immune Facet

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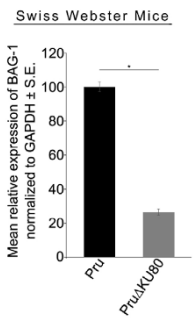
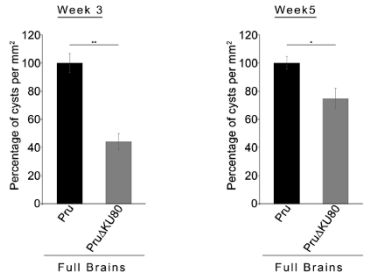
Background: *Toxoplasma gondii (T.gondii)* is the causative agent of toxoplasmosis, a fatal disease in the immunocompromised. It hijacks macrophages to travel to host sites, evading complete clearance and establishing chronic toxoplasmosis inside cysts in the hosts' brain. Type II *T. gondii* strains are mostly isolated from patients. A Pru strain derivative, Pru*ΔKU80,* was generated to ease efficient gene knock-outs. Differences in their capacity to form brain cysts, influenced by the host immune system, has never been tested, thus we addressed this question in Swiss Webster mice.

Design: Pru and Pru*ΔKU80* strains were grown in Human Foreskin Fibroblasts. 10 mice per strain were infected by 100 parasites each. Day 7 post infection (pi) serum was used as primary antibodies against *Toxoplasma* proteins to verify acute toxoplasmosis. 4 weeks pi, full brains of 5 mice per strain were harvested and sectioned in the coronal plane at a thickness of 5 mm, embedded in paraffin, stained with haematoxylin-eosin, then examined by light microscopy. The numbers of bradyzoite cysts and microglial nodules per mm^2 were assessed in each brain region. The brains of the other 5 mice were harvested, full RNA was extracted, and RT-PCR was performed using primers specific for BAG-1, a bradyzoite marker, and various cytokines.

Results: BAG-1 transcript levels were lower upon infection with Pru*ΔKU80.*



Pathological findings showed lower cyst counts per mm^2 in Pru*ΔKU80* at weeks 3 and 5 pi.



These results correlated with lower brain expression of the different cytokines transcripts in Pru*ΔKU80* infected mice as compared to Pru. Hence, Pru*ΔKU80* has a weaker capacity to modulate the host immune system leading to less cysts in the brain.

Conclusions: We present the first report investigating *in vivo* properties of two widely used strains for understanding toxoplasmosis biology, thus alarming researchers on the use of either strain according to the addressed hypothesis.

1568 Cystoisospora Infection of Gallbladder in Immunocompetent Patients: A Study of 1000 Consecutive Cholecystectomy Specimens

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Background: *Cystoisospora belli* is an obligate intracellular coccidian parasite which causes self limited diarrheal illness in immunocompetent patients. However,it can cause chronic life threatening diarrhea in immunocompromised. This infection is under-recognized and has recently been reported in cholecystectomy specimens from immunocompetent patients. On consecutive cholecystectomies in immunocompetent patients, this is the largest study with an aim to determine the incidence and clinico-pathological characteristics of Cystoisospora infestation of gallbladder.

Design: Four pathologists reviewed 1000 consecutive cholecystectomy specimens for Cystoisospora parasitic forms (798 females, 202 males; 821 with cholelithiasis, 179 acalculous). Histologic mimics of Cystoisospora were excluded, and PAS-D, trichrome and AFB were performed in equivocal cases to confirm the diagnosis. Only immunocompetent patients were included, and detailed clinical and histopathologic analysis was performed.

Results: We identified 14 patients with Cystoisospora, and none of these patients had chronic diarrhea, malabsorption or clinical suspicion for intestinal Cystoisospora infection. The average follow up period was 14 months. After surgery, all patients were asymptomatic except one who developed abdominal pain and diarrhea after 2 months and lost to follow up. Also, we identified microsporidum in two cases.

Parameter	Gallbladder With Stones (n=9)	Gallbladder Without Stones (n=5)
Age (yr) Median Range	40.129-59	38.824-49
Gender Female Male	72	50
Presentation Symptomatic stones Acute cholecystitis Biliary dyskinesia Acute pancreatitis	8100	0041
Inflammation Acute Chronic Absent + eosinophils	4323	0322
Wall thickening on imaging	3	0
Distribution of organisms Diffuse Focal	09	23

Conclusions: Cystoisospora infection is under-recognized in cholecystectomy specimens. In our cohort, Cystoisospora was identified in 1.4% of cholecystectomies (2.7% in acalculous cases). A high index of suspicion is required to identify these organisms as the infection might be focal and limited to the cystic duct. The possible etiologic role of Cystoisospora in biliary dyskinesia needs to be further investigated as it may play a role in treatment planning including a trial of antibiotic therapy prior to decision for surgical removal.

1569 Granulomatous Inflammation Diagnosed by Fine Needle Aspirate Biopsy and Ancillary Testing

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Background: Fine needle aspiration biopsy (FNAB) is useful in the diagnosis of infectious disease as a minimally invasive technique that allows for cytologic evaluation and ancillary testing. Granulomatous inflammation (GI) may be present in the setting of infection, sarcoidosis, foreign body reaction and malignancy. Given the broad differential diagnosis, tissue sampling in an effective and safe manner by FNAB is often critical.

Design: We searched the pathology database for FNAB with diagnoses of "granulomatous inflammation" and "granuloma" from 1/1/2000 to 3/1/2016. Cases were classified as necrotizing granulomatous inflammation (NGI) or non-necrotizing granulomatous inflammation (NNGI) and correlated with clinical history, special stains (Kinyoun and Grocott's methenamine silver stains) and culture results, when available.

Results: 339 FNABs with GI were identified, representing 306 patients (average age 47 years; 160 females, 146 males) with 24 patients receiving at least 1 repeat FNAB. There were 117 cases with NGI, 222 with NNGI. Specimen sites, with number of cases, included: lymph nodes (200), lung (56), soft tissue (44), breast (12), salivary gland (10), liver (8), thyroid (4), kidney (2), pancreas (1), spleen (1), brain (1). Microorganisms were detected in 103/339 (30%) FNABs by morphology, special stains, and/or culture;

NGI was seen in 53/103 (51%) and NNGI in 50/103 (49%) positive specimens. Culture results were available in 239 FNABs: 40 were positive for *Mycobacterium tuberculosis* complex (MTC), 14 for atypical mycobacteria, 6 for *Coccidioides immitis*, 2 for *Histoplasma capsulatum*, 2 for *Penicillium marneffei*, 1 case each for *Aspergillus spp.*, *Escherichia coli, Nocardia spp., Staphylcoccus aureus* and unclassified acid-fast bacilli. Fungal organisms were detected in 21/339 (6%) cases by cytology, special stains, and/or culture; fungal organisms were morphologically noted in 18 samples. Mycobacteria comprised 76/339 (22%) samples; 39/76 (51%) were positive by culture only, and 20/76 (26%) by Kinyoun stain only. Malignancy was seen in 4/339, and foreign body reaction in 16/339 specimens. Sarcoidosis was suspected in 17/339 cases.

Conclusions: Granulomatous inflammation has a wide range of causes including infection and malignancy. FNAB is a safe, minimally invasive technique amendable to various organ sites that allows for cytomorphologic diagnosis and ancillary studies such as special stains, culture and molecular testing. FNAB can be performed with minimal risk of contamination and is useful for the evaluation of GI when correlated with clinical history and ancillary testing.

1570 Jones' Methenamine Silver Stain Is a Useful Adjunct Stain in the Histopathologic Diagnosis of Leishmaniasis

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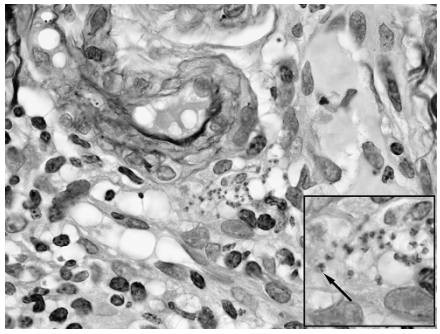
Background: Leishmaniasis (cutaneous or visceral infection by *Leishmania spp.)* is a challenging diagnosis in formalin-fixed paraffin-embedded tissues due to the small size of *Leishmania* amastigotes (2-4 micrometers in diameter). Definitive diagnosis is typically made from H&E-stained tissues, and relies on the identification of key morphologic features: the nucleus and rod-shaped kinetoplast. Differential diagnoses includes cellular debris and yeasts (e.g. *H. capsulatum* or *C. glabrata*). H&E staining is usually sufficient for diagnosis of *Leishmania*. However, anecdotal reports suggest stains such as Giemsa, Twort's Gram stain, silver oxidation-reduction stains (GMS, Jones' Silver, Reticulin) and argyrophil stains (Warthin-Starry, Steiner, Dieterle) may assist in *Leishmania* diagnosis in some difficult cases. Therefore, a review of *Leishmania* cases was performed to determine if diagnosis of difficult cases could be enhanced by special stains.

Design: Eight cases of cutaneous Leishmaniasis with available tissue blocks were identified. The cases were reviewed, and two cases considered "marginal" (potentially difficult to diagnose on H&E alone) were selected. Additional slides from those cases were stained with H&E, Gram, Giemsa, PAS, GMS, Jones' Silver, Reticulin, Steiner, and Warthin-Starry stains. All special stains were compared with H&E for their ability to highlight amastigote features (e.g., nucleus and/or kinetoplast), and background (non-specific or off-target) staining.

Results: H&E with oil immersion (1000X) was sufficient for diagnosis in all eight cases. In the two marginal cases, Jones' Silver stain (with H&E counterstain) provided an improvement over H&E in identification of the amastigote kinetoplast, with little additional background staining.



Twort's Gram stain, PAS, and Giemsa staining did not subjectively improve upon H&E for identification of amastigote features. Warthin-Starry, Steiner and Reticulin stains had far too much background staining to be useful diagnostic aids.

Conclusions: Jones' Silver stain highlights Leishmania amastigote kinetoplasts in skin with minimal background staining, and may be a useful adjunct stain in the histopathologic diagnosis of Leishmaniasis.

1571 Expression of Platelet-Associated Marker CD42b on Histoplasma

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Background: In pathologic specimens, *Histoplasma capsulatum*, can be readily identified by morphology and special stains such as GMS or PAS. In most cases, it is rarely necessary to use more complicated techniques, although PCR and serologic methodologies exist.

While evaluating a bone marrow biopsy infected with histoplasmosis, we identified staining using the platelet associated marker CD42b, also known as glycoprotein (GP) Ib. It is a robust immunohistochemical stain, allowing easy identification of a megakaryocytes, megakaryoblasts and platelets. The unusual staining appeared to be on the surface of fungi, highlighting them from the background.

As a result, we evaluated multiple cases with histoplasmosis using CD42b staining to determine if there was reaction in the fungal organisms.

Design: 13 cases were obtained from multiple institutions. Tissues were from lymph node (6), lung (3), soft tissue (3), and bone marrow (1). Original diagnoses of *Histoplasma* infection were confirmed by histologic evaluation and special stains. Immunohistochemical staining for CD42b was performed in each case and compared to a GMS stain in the same tissue. In one case, staining was also performed megakaryocyte/platelet markers, CD61 and Factor VIII.

Results: We found high concordance between the results of a GMS stain and the CD42b staining in fungal organisms (9/13). In several cases, more organisms and larger numbers of possible organisms were identified using the CD42b stain. In three cases, staining for putative organisms was identified with no staining for GMS. In one case, no staining was identified on CD42b, but GMS positive organisms were in an area of tissue fall-off. In some cases, we found black or brown pigment (hemosiderin, lipofuscin) which complicated GMS interpretation. In the CD42b stain, positive results could be easily distinguished from the black pigments. No staining was identified in organisms for Factor VIII or CD61.

Conclusions: We found strong and reproducible staining in *Histoplasma* organisms using CD42b, a platelet marker. Recent studies have shown that a distinctive fungal molecule, fusicoccin, which can interact with the GPIba. This interaction of fusicoccin stabilizes the platelet protein complex formed by GPIb-Factor IX and Factor V. This may suggest that there is a molecular basis for the immunohistochemical findings. Finally, this interesting staining could be diagnostically useful to identify cases of histoplasmosis with challenging morphologic findings.

1572 Immunohistochemical Study of Mucins in Human Intestinal Spirochetosis

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Background: Most patients with human intestinal spirochetosis (HIS; a colorectal bacterial infection caused by *Brachyspira* species) seem asymptomatic, and some commentators consider it harmless. Recently, alterations in mucin expression were reported in animal *Brachyspira* infection. The present question was "Is mucin expression altered in HIS?".

Design: Using antibodies for MUCs 1, 2, 4, 5AC, and 6, we immunohistochemically examined 215 specimens from 83 histology-confirmed HIS cases. Positive staining (which included even focal positive staining on the epithelium) was rated "high (+)" or "low (+)". Results were analysed for four categories of lesions, and associations between MUC expression and spirochetal presence were also analysed.

Results: In the normal/inflammation category: high (+) of MUC2-positivity was more frequent in HIS than in control. In the hyperplasia/serrated polyp category: in HIS (vs. control), the MUC5AC-positivity rate was lower, while high (+) of MUC4-positivity was more frequent. In the conventional adenoma category: in HIS (vs. control), the MUC1-positivity rate was lower, while both high (+) MUC2-positivity and high (+) MUC5AC-positivity were less frequent. In the adenocarcinoma category: high (+) MUC2-positivity was more frequent in HIS than in control. Among the above mucins, only MUC1-positivity was significantly associated with an absence of the so-called fringe formation, an absence of spiral organisms within mucus, and an absence of strong immunopositive materials within the epithelial layer and within the subepithelial layer.

Conclusions: The results suggest that *Brachyspira* infection may alter the large intestine mucin-expression profile in humans, and simultaneously suggest that *Brachyspira* may not be innocent or commensal bacteria in humans.

1573 Minimally Invasive Autopsy for Cause of Death Determination in Stillbons and Neonates

Jaume Ordi, Paola Castillo, Miguel J Martinez, Dercio Jordao, Lucilia Lovane, Carla Carrilho, Mamudo R Ismail, Cesaltina Lorenzoni, Jordi Vila, Quique Bassat, Clara Menendez. Institute for Global Health (ISGloblal), Hospital Clinic, Universitat de Barcelona, Barcelona, Spain; Hospital Central de Maputo, Universidade Eduardo Mondlane, Maputo, Mozambique.

Background: Performing complete autopsies (CA) is challenging in low and middle income countries due to lack of resources, the large proportion of deaths occurring outside the health system, and cultural and/or religious apprehension. We aimed to evaluate the validity of the minimally invasive autopsy (MIA) to determine the CoD in a series of stillbirths and neonatal deaths occurring in Mozambique, by comparing the MIA-putative diagnosis with the gold standard CA-diagnosis.

Design: Coupled MIA and CA were performed to 18 stillborns (late fetal deaths, gestational age >28) and 41 neonates who died at the Maputo Central Hospital. The MIA procedure involved the collection of blood and cerebrospinal fluid (CSF) and puncture of liver, lungs, and brain using biopsy needles. Histological and microbiological examination of the MIA samples was done blindly, without any knowledge of the clinical data or the results of the CA. We compared the putative-MIA and the final-CA diagnoses.

Results: A final-CA diagnosis was identified in 16/18 (88.9%) stillborns. The CoDs in this group were fetal growth restriction (7 cases, 38.9%), infectious diseases (4, 22.2%, with 2 group B *streptococcus*), intrapartum hypoxia (3, 16.6%), and intrauterine hypoxia (2, 11.1%). A MIA-putative diagnosis of CoD was identified in 15/18 (83.3%) stillborns. The MIA-putative and the final CDA-diagnoses agreed in 83.3% of the cases and showed a substantial concordance (Kappa = 0.78, p-value < 0.001). A final-CA diagnosis was identified in 40/41 neonates (97.6%). The CoDs in this group were infectious diseases (26 cases, 63.4%; 19 neonatal sepsis, 7 non-neonatal sepsis, with 1 congenital cytomegalovirus and 2 congenital Herpes virus 2); pre-term complications (5, 12.2%); congenital malformations (4, 9.8%); birth asphyxia due to intrapartum related conditions (3, 7.3%); and other conditions (2, 4.9%). A MIA-putative diagnosis was identified in 34 (82.9%) of the neonates. The putative-MIA and the final-CA diagnoses agreed in 68.3% and showed a moderate concordance (Kappa = 0.43, p-value < 0.001) in this age group. The concordance was higher for infectious diseases (84.6%). The MIA identified the specific microorganism causine death in 86.9% cases.

Conclusions: The MIA approach may provide unique information on the causes of mortality of stillborns and neonates. This approach could help at saving global health funds by guiding them to be spent on the most effective health programs.

1574 Acid Fast Bacilli Status on Non Gynecologic Cytology Specimens, a Study from Colombia

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Background: Colombia has a high incidence of tuberculosis, and we routinely perform AFB special stain on pulmonary non-gynecological cytology specimens or other locations with high lymphocyte count. The frequency of ZN positivity among the literature varies between 10-30%. However its frequency in non gynecological cytology specimens have not been described.

Design: We performed a retrospective review from 2010 to 2015 database for AFB stains done on non gynecological cytology specimens. Some samples were processed by cytospin (CS) and some by liquid based cytology (LBC). Data were collected and clinical records were searched in order to correlate AFB result with culture, PCR and technique used for processing when available.

Results: We found a total of 2322 non gyn samples that included 1070 BAL, 601 pleural fluids, 454 ascitis and 197 other type of fluids. 1108 were processed by CS and 1241 were processed by LBC. A total of 469 (42%) CS AFB and 602 (48%) CBL AFB were done. AFB was performed in 46% of samples, we found 1.89% (n=44) of true positive cases for Mycobacterium Tuberculosis or Complex confirmed by culture and PCR. A 0.4% (n=9) of the negative AFB cases was positive for M.Tuberculosis by PCR and culture (Falsos negativos)

16 AFB CS (4%) and 28 AFB CBL (5%) were positive. 37% CS Vs 25% CBL + AFB were concordant by culture and PCR. 31% CS VS 39% CBL + AFB were negative by culture and PCR. 18 % CS Vs 7% LBC AFB+ had negative culture and positive PCR, probably due to poor viability of the sample.

	CS CULTURE			LBC CULTURE		
	+	-	NA	+	-	na
PCR +	6	3	-	7	2	4
PCR -	-	5	1	-	11	-
NA	-	1	-	-	-	-

Among AFB negative cases 8 positive culture and PCR confirmed were identified and 2 possible contamination

	CULTURE CS			CULTURE LBC		
	+	-	NA	+	-	NA
PCR +	1 (FORTUITUM)	-	-	4	-	-
PCR-	2	2	-	4 (SMEG, ABSC, TBC, M COPLEX	-	-
PCR NA	4	1	-	-	-	-

Conclusions: AFB stain was able to detect 44 cases (1.89%) when performed on non gyn cytology specimens. Ability to detect positive Mycobacteria was similar when cytospin technique was compared to Liquid based cytology. False positive AFB are usually explained by other non mycobacterial AFB positive microorganisms such as Nocardia.

1575 RNA ISH for Epstein-Barr Virus and Cytomegalovirus: Comparison with In Situ Hybridization and Immunohistochemistry

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Background: The RNAscope utilizes in situ hybridization (RISH) technology to detect single RNA molecules from virtually any gene in a variety of tissue samples, including formalin fixed paraffin embedded (FFPE) tissues. The sensitivity and specificity of RISH has been previously described. Epstein-Barr virus (EBV) and cytomegalovirus (CMV) are found in association with neoplastic tissues and other lesions, necessitating the use of immunohistochemistry (IHC) or other techniques (ISH) to identify them. We compared the RNAscope RISH to ISH and IHC in the detection of EBV and CMV respectively to provide a reference point for the usage of the RNAscope in a clinical setting and how it compares to other techniques.

Design: 31 FFPE tissues were stained by RISH to detect EBV and 24 samples of tissue for CMV from carcinoma (10 for EBV, 20 for CMV) and lymphoma (21 for EBV, 4 for CMV) from nasopharynx to rectum. The RISH used the RNAscope (Leica BioSystems, Buffalo Grove, IL), the Bond III autostainer (Leica), and probes V-EBV and V-CMV (Advanced Cell Diagnostics, Newark, CA) as well as negative (DapB) and positive probe (PPIB) for RNA. Results were compared with those by ISH (Leica, EBV RNA probe), and IHC (Dako, 1/160), respectively.

Results: The concordance between RISH and ISH interpretation was 100% in cases determined to be positive for EBV by ISH (19/19). Of the cases determined to be negative for EBV by ISH, RISH interpretation detected positive cells in an additional 25% of the samples (3/12). Overall concordance was 90.3% (28/31). The concordance between RISH and IHC interpretation was 100% in cases determined to be positive for CMV by IHC (8/8). Of the cases determined to be negative for CMV by IHC, RISH interpretation detected positive cells in an additional 50% of the samples (8/16). Overall concordance was 66.7% (16/24). Three pathologists reviewed the RISH slides and consensus was achieved on all interpretations.

	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Accuracy
EBV (31)	100%	75%	86.4%	100%	90.3%
CMV (24)	100%	50%	100%	50%	66.7%

Conclusions: RISH staining for EBV and CMV are easy to interpret because of low background and strong positive stain. The results for both EBV and CMV RISH demonstrate complete concordance with cases positive by ISH and IHC respectively. RISH demonstrates increased sensitivity in the clinical setting, especially for CMV, detecting positive cells not stained by ISH and IHC and thereby interpreted as negative.

1576 Frozen Sections Are Unreliable for the Diagnosis of Necrotizing Soft Tissue Infections

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Background: Necrotizing soft tissue infections (NSTI) are rare but severe infections of subcutaneous tissue associated with high rates of morbidity and mortality. Rapid diagnosis and definitive treatment with antibiotics and surgical debridement have been shown to be the most important factors in survival. Lack of experience can lead to unnecessary delays in diagnosis and treatment. Numerous clinical, radiologic, and laboratory tests have been investigated for their utility in this setting. Frozen section evaluation has been proposed as a method to achieve earlier diagnoses and better outcomes; however, no large studies have been published to determine the sensitivity and specificity of frozen section histologic findings for the diagnosis of NSTI.

Design: All biopsies with frozen section evaluation for NSTI at a large academic medical center were identified over a 20-year period. Frozen section slides were independently reviewed by three pathologists and assessed for the number of neutrophils, extent of necrosis, presence of thrombi, bacteria, karyorrhexis, and fibrin, and concordance with permanent sections. Histologic findings were compared with final clinical diagnosis (gold standard) and culture results.

Results: From 1995-2015, 175 surgical pathology cases included frozen section evaluation to assess for NSTI (166 available for review). NSTI was clinically diagnosed in 60/166 (36%) patients: 5 with *Staphylococcus aureus*, 15 with *Streptococcus pyogenes*, 34 polymicrobial or other single species, and 8 with negative cultures. Compared to negative cases, biopsies with NSTI were more likely to be associated with >25 neutrophils/HPF (82% vs 49%), focal or extensive necrosis (71% vs 31%), presence of thrombi (31% vs 12%), bacteria (13% vs 2%), karyorrhexis (74% vs 38%), and fibrin (75% vs 39%). Frozen sections were 82% sensitive for detecting at least one histologic feature associated with NSTI, but only 40% specific (i.e. negative cases with zero features). Concordance between frozen and permanent sections was 92%, with only 13 cases showing foci of neutrophils or bacteria on permanent but not frozen sections.

Conclusions: Frozen sections in cases of NSTI most often show abundant neutrophils, fibrin, karyorrhexis, and necrosis, less often thrombi, and rarely bacteria. Negative cases were associated with all of the same features in lower frequencies. These findings suggest that frozen sections are likely to be of limited clinical utility due to the high false negative rate (18%) and low specificity (40%).

1577 Mycobacteria Immunohistochemistry Is an Effective Screening Tool for Molecular Identification

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Background: Mycobacterial infections cause significant morbidity and mortality worldwide. Cultures for identification and antibiotic susceptibility testing can take weeks to months due to the slow-growing nature of the organisms. Molecular tests for mycobacteria allow for significantly faster identification and more rapid implementation of appropriate treatment for species that have empiric guidelines. Due to the relative rarity of mycobacterial infections, potential for detection of nonpathogenic environmental contaminants, and substantial costs associated with molecular diagnostics, effective screening methods are needed to identify samples most suitable for molecular testing. Anatomic pathology specimens can be utilized to identify characteristic histologic patterns and to directly visualize mycobacteria through AFB stains (e.g., Ziehl-Neelsen). The utility of immunohistochemistry (IHC) to identify mycobacteria in this setting is unknown.

Design: All anatomic pathology cases tested for mycobacteria by PCR at a large academic medical center were retrospectively identified. IHC was performed with an anti-mycobacterial antibody (Biocare CP140). IHC results were compared with histology, AFB stain, PCR and culture results.

Results: From 2009-2016, 143 cases underwent testing for mycobacteria by PCR, and 121 cases (111 surgical cases and 10 cytology cell blocks) had material available for IHC. Mycobacterial DNA was detected in 54 cases: 12 *Mycobacterium tuberculosis* (*MTB*) and 42 nontuberculosis mycobacteria (NTM), while the remaining 67 cases were negative. Concurrent cultures were positive in 40/87 (46%) cases, which confirmed the diagnosis of 5 *MTB* and 11 NTM cases. Necrotizing granulomas were present in 75% *MTB*, 69% NTM, and 75% negative cases. AFB stains were positive in 50% MTB, 92% NTM, and 57% negative cases. IHC was positive in 50% MTB, 81% NTM, and 49% negative cases. Organisms were identifiable by IHC using a 10x objective in the majority of cases. Concordance between IHC and AFB stains was 91%, with discordant results attributed to lack of lesional tissue on the IHC levels in 9 cases. Negative PCR with positive IHC was attributed to paucity of organisms in 30/33 cases.

Conclusions: IHC has similar sensitivity as AFB stains for detecting mycobacteria; however, IHC slides are significantly easier to review since organisms can be visualized

using a 10x objective. IHC is highly sensitive for NTM but has a lower sensitivity for *MTB*, suggesting that cases with a high clinical suspicion for *MTB* should be sent for PCR even when AFB and IHC are negative.

1578 Morphologic Characterization of Incidentally Discovered Granulomas in Lung Neoplasms

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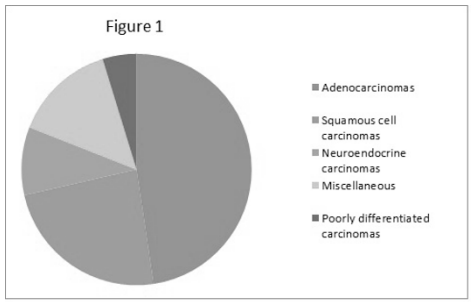
Background: Granulomatous reaction to tumor is an unusual phenomenon and has been described within main tumor or in the draining lymph nodes. Lung is commonly involved by granulomatous lesions of various infectious and non-infectious etiologies. Co-existence of granulomatous inflammation in lung tumors may pose diagnostic difficulties and may influence therapeutic decisions. The aim of the study is to identify and describe the morphological patterns of granulomatous inflammation seen in association with lung neoplasms

Design: This single institutional retrospective study from 2001 to 2015 identified 42 lung resection specimens of lung cancers with coexisting granulomatous inflammation.

Results: The median age of the patients was 58 years of whom 65.2% were males. Majority of the cases were lobectomies (n=40); followed by pneumonectomies (n=2). All 42 cases had granulomas within the adjacent lung parenchyma. None of the cases elicited a granulomatous response within tumor stroma. Of these, 5 cases also had granulomatous involvement of the draining lymph nodes without evidence of metastasis. Several morphologic types of granulomas were encountered: Hyalinized (45.2%), calcified (23.8%) and necrotizing (31%). GMS stain identified Aspergillus and Histoplasma as the infectious cause of necrotizing granulomas in 2 of the cases. AFB stain was negative in all the cases. The distribution of the granulomas across subtypes of lung neoplasms is depicted in figure 1.



Tumors metastatic to the lung were included under miscellaneous.

Conclusions: In our study set across 15 years, we found that most of the granulomas associated with lung neoplasms are noted to be of non-infectious etiology. It is not clear whether granulomas in association with lung neoplasms and draining lymph nodes, are of any prognostic significance; It is likely that these may represent an aberrant immunological response to tumor related antigens. Being in a histoplasma endemic region, the infectious etiology in necrotizing granulomas still has to be ruled out by PCR testing.

1579 Primary Syphilis in the Tonsil: Challenges in Histopathological Diagnosis

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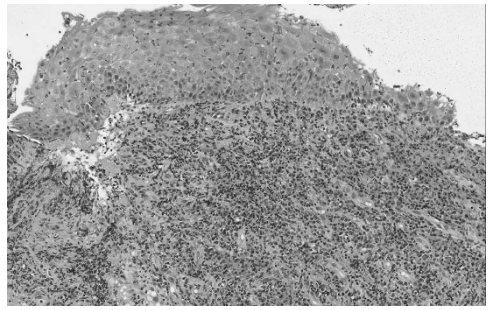
Background: Primary syphilis usually appears on the genitalia, but may present on other sites such as anus or oral cavity. There have been 12 cases of tonsillar primary syphilis published in English literature. Most of them had sufficient clinical information prior to the diagnostic tests, and detailed pathological information was not provided. We describe an additional case with challenging pathological diagnosis due to lack of clinical information.

Design: We report a 30-year-old man with the chief complaint of sore throat for one week. His right tonsil showed grade IV swelling with an ulcerative surface. A biopsy was performed. The tissue was formalin-fixed and paraffin embedded. Hematoxylin and eosin stained slides were reviewed and further immunohistochemical studies were performed.

Results: Microscopically, the tonsillar tissue showed ulcerated squamous epithelium with fibrinopurulent exudates and reactive keratinocytic atypia. The underlying connective tissue and lymphoid nodules showed heavy infiltrations of plasma cells, lymphocytes and histiocytes. Perivascular plasmalymphocytic infiltrations and endarteritis are seen. Immunohistochemically, there was no light chain restriction by kappa and lambda immunostains. The plasma cells are diffusely positive for IgG, but only focally positive for IgG4. Periodic acid-Schiff stain and acid-fast stains showed no fungal or mycobacterial infection. Finally, numerous *Treponema pallidum* were detected by anti-*T. pallidum* antibody in the squamous epithelium, submucosa, and vessel walls. After pathologically diagnosis of a primary syphilis, serologic tests were performed. The Rapid Plasma Reagin test was reactive at 1:128, and the *Treponema Pallidum* Particle Agglutination test showed reactive at > 1:1280. As a result, the diagnosis of primary syphilis was confirmed.



Conclusions: Pathological diagnosis of primary syphilis may be challenging without sufficient clinical information, because the microscopic features are not specific. A tentative diagnosis can be made by combination of clinical information and microscopic findings, and serologic tests are needed for confirmation.

Informatics

1580 The Automatic Extraction and Categorization of 11,347 Large Bowel Polyps from 54,631 Free Text Surgical Pathology Reports

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Background: Observational data is commonly used in process management in clinical pathology and manufacturing; however, anatomic pathology data related to pre-cancerous conditions is not routinely assessed to extract trends and gauge the variation due to health care providers.

Design: All surgical pathology reports (54,631) for a 3 year period, in a large academic center, were data-mined using a custom computer program to extract all large bowel polyp specimens. Diagnostic information including the anatomic location was categorized using keyword searches, an approximate string matching library (google-diff-match-patch), and hierarchical pruning. The categorizations by the computer were compared to pathologist reads for a random subset of reports to assess accuracy.

Results: 53,967 (98.8%) of the complete reports were successfully parsed into individual specimen parts, and 11,457 large bowel polyp specimens were extracted. 68 cases with large bowel polyps could not be parsed, due to unusual report formatting (37), mislabelled parts (24), and missing specimens parts (7). 11,347 (99%) of large bowel polyps were diagnostically classified, and human reads of a randomly selected subset of 200 specimens demonstrated that the computer's diagnostic categorization was correct >98% of the time. Using the parsed output, the tissue acquired and diagnostic interpretation were stratified by the submitting endoscopist and the signing pathologist using a spreadsheet (Microsoft Excel). Completeness of the clinical history could be stratified by submitting endoscopist. The program could successfully parse and extract a large number of reporting styles; however, it failed more frequently when parts were lumped or when the reporting style significantly deviated from Association of Directors of Anatomic and Surgical Pathology recommendations.

Conclusions: The automatic extraction and categorization of diagnostic information in free text pathology reports is feasible and yields a large quantity of information that can be easily analysed. Pathologist reporting style may be a modifiable factor that could further improve analyses of this type. Routine analyses of observational data for pre-cancerous conditions may facilitate (1) a greater understanding of the pathobiology in the served population, (2) factors important for optimization of care, and (3) improved cancer prevention and early cancer detection.

1581 A Synoptic Electronic Order Set for Placental Pathology: A Framework Extensible to Non-Neoplastic Pathology

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Background: Synoptic reporting templates in neoplastic specimens are deemed crucial for quality patient care through comprehensive pathology reporting.Overlooked however is how pathology reporting suffers when there is omission of important clinical information prior to the pathologic evaluation.Particularly with non-neoplastic specimens like placental, renal, and liver;availability of certain curated clinical data elements are critical to obtaining the most accurate diagnosis.For instance historically paper requisitions in placental pathology allow for omission of clinical data or vague over-generalized histories such as "intrauterine pregnancy",which add nothing to help the pathologic assessment.

Design: With widespread adoption of electronic medical records,opportunities arise for electronic order sets (EOS) that ensure capture and availability of discrete clinical data.Currently however,no frameworks for EOS exist in anatomic pathology.Thus through clinical and pathologic curation via collaboration between the clinical teams and pathology,we have constructed an EOS for placental specimens.Many data elements are optional but included in our EOS are mandatory and critical data elements.