337 Left Ventricular Assist Device Can Increase Capillary Density of Human Heart

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Background: Prolonged ventricular unloading with a left ventricular assist device (LVAD) or other continuous flow devices have been used significantly in recent years to treat severe heart failure. The use of LVAD has been shown to lead to myocardial recovery in some patients, indicating that some degree of cardiomyocyte regeneration can be induced by decreasing the external load. Furthermore, mitosis and cytokinesis were reported to increase significantly in the post-LVAD hearts. This study is aimed to determine the cellular response of human heart to the prolonged mechanical unloading by a LVAD.

Design: Pre- and post-LVAD heart tissue samples were collected from 11 patients at the time of LVAD implantation and heart transplantation. The 11 patients included 2 females and 9 males with age ranging from 27 to 71 and averaging 57. Four patients had ischemic cardiomyopathy and seven patients had non-ischemic cardiomyopathy. All had no histologic evidence of active myocarditis. The duration of LVAD ranged from 1.5 to 56 months with an average of 10 months. The tissue was processed in the histology lab of Temple University Hospital for H&E and immunostaining. The capillary density was measured by ImageJ software on the CD31 immunostained slides. The pre- and post-LVAD samples were compared using the Wilcoxon signed rank sum test. The mRNA levels of basic fibroblast growth factor (bFGF) were measured by quantitative RT-PCR from paraffin-embedded formalin-fixed tissue.

Results: Of the 11 matched pre- and post-LVAD tissue samples, 10 demonstrated an up to 167.1% increase in capillary density after the use of LVAD (range= 20.0-167.1%, 66.3+/49.5%). One showed a 30.1% decrease in capillary density (duration of LVAD=10 months). The increase of capillary density in post-LVAD cardiomyocytes is statistically significant (p=0.0098). The amount of changes in capillary density was not related to the duration of LVAD (p=0.69). There were no significant changes in the amount of bFGF mRNA between pre- and post-LVAD groups.

Conclusions: Significant positive effects on the vascular architecture of human heart were observed by the prolonged ventricular unloading of LVAD. This improved vascularization of cardiomyocyte may play a role in the switch from a hypertrophic state to a hyperplastic state induced by decreasing the external load of the human heart.

338 Diagnostic Value of T helper Type 17 (Th17) Cells in Moderate Acute Cellular Rejection of Cardiac Allograft

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Background: A diagnosis of moderate acute cellular rejection (grade 2R) is clinically important since it triggers adjustment in the immunosuppressive regimen whereas mild acute cellular rejection (grade 1R) usually does not. However, myocyte damage, a required morphologic feature for diagnosis of grade 2R rejection can be difficult to ascertain since the morphologic spectrum of myocyte damage is wide and has subtle changes. Studies have shown a major source of discordance in the grading is the criteria used for the interpretation of myocyte damage. Thus, it is necessary to explore new ancillary studies such as immunohistochemistry to improve accuracy of the morphologic diagnosis of grade 2R rejection. Recent research data suggest that the IL-17 producing T helper type (Th17) cell plays a crucial role in the acute allograft rejection.

Design: 25 cases of cardiac transplant biopsies with moderate acute cellular rejection were found in archives of Calgary Laboratory Services. The immunohistochemical stain for IL-17 was performed on the paraffin sections of the above biopsy specimens. The previous cardiac biopsy with diagnosis of mild acute cellular rejection from the same patient with the moderate acute cellular rejection was used as control. The Th17 cells were counted in the lymphocytic infiltrate regions. The data are expressed as number of cells per high power field (HPF).

Results: As compared with mild acute cellular rejection, biopsies with moderate acute cellular rejection had significantly increased number of Th17 cells (2.99 ± 2.29 cells/HPF vs 1.14 ± 1.50 cells/PHF, $p<0.001$).

Conclusions: The Th17 cell infiltration in the cardiac allograft is associated with severity of cardiac acute cellular rejection. Evaluation the number of Th17 cells in the cardiac transplant biopsies might be helpful in assistance with diagnosis of moderate acute cellular rejection and improve diagnostic accuracy of moderate acute cellular rejection.

339 Beta Amyloid Precursor Protein in Carotid Artery Plaque Macrophages: Correlation with Features of Plaque Stability

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Background: Beta amyloid precursor protein (bAPP) is the amyloidogenic protein present in amyloid deposits of Alzheimer plaques and cerebral amyloid angiopathy. Its native function and activity (when properly folded and not produced in excess) are not fully understood, however bAPP is found in platelets and has been implicated in vascular inflammation and atherosclerosis. The aim of this study was to determine whether native bAPP (not amyloid) could be found in carotid plaques and if its presence or localization correlated with features of plaque instability.

Design: Intact carotid endarterectomy specimens were obtained as part of an ongoing IRB approved radiology-pathology collaborative study. The samples were formalin fixed and decalcified. H&E and elastic stains were performed to allow for characterization of plaques and features of instability (soft/necrotic atheroma, intraplaque hemorrhage, thin fibrous cap, cap inflammation). Immunoperoxidase staining for bAPP (DAKO clone 6F/3D) was also performed and the stained sections reviewed. The localization of staining was recorded along with a semiquantitative assessment of staining intensity and abundance. Immunohistochemistry data was correlated with features of plaque instability using the Pearson correlation method.

Results: 55 carotid tissue blocks from 35 patients comprised the sample population. The mean patient age was 69 years and 3 were women. 12 had ipsilateral strokes. 35 (64%) of the plaques showed features of instability. bAPP staining was limited to macrophages in a coarse granular cytoplasmic pattern, consistent with the reported phenomenon of macrophage phagocytosis of platelets expressing bAPP. These cells were present in and around the plaque cap and in areas of plaque hemorrhage. For unstable plaques, the mean semiquantitative bAPP score was 1.9 and for stable plaques it was 0.6 ($p<0.0001$) Correlation coefficients were determined for semiquantitative bAPP scoring versus percent of soft/necrotic plaque component ($r=0.57$, $p<0.0001$), thickness of fibrous cap ($r=0.14$, $p=.31$), and cap inflammation ($r=0.34$, $p=0.012$).

Conclusions: This preliminary study is the first to show accumulation of non-amyloid bAPP within carotid plaques and demonstrate an association with at least some specific features of plaque instability. Whether bAPP accumulation has mechanistic significance beyond plaque inflammation and instability remains to be determined and needs further study.

340 Implementation of the New AECVP/SCVP Aorta Consensus Grading Scheme

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Background: The Association for European Cardiovascular Pathology (AECVP) and the Society for Cardiovascular Pathology (SCVP) have developed a new consensus nomenclature and grading scheme for degenerative, noninflammatory thoracic aortic specimens in order to improve the consistency of diagnosis for this specimen type. We tested the usability of these new terms on routine surgical pathology aorta cases.

Design: 100 consecutive noninflammatory aortic specimens and basic phenotypic data were obtained from the surgical archives. After an initial general review of aortic histopathology with two trainees and one cardiovascular pathologist, each case was independently scored by two observers in a blinded fashion for 13 features using H&E and Movat stained slides. All data was categorized, converted into numbers, tabulated and analyzed using a Mann Whitney U test.

Results: There was strong overall consensus on the overarching diagnosis of medial degeneration with only 3 of 100 cases demonstrating two levels of disagreement. There was more variability in the usage of less common findings such as elastic fiber thinning. Forty-seven cases had a known genetic syndrome (bicuspid aortic valve (BAV), Marfan, Loeys Dietz, FTAAD), with an average age of 42. The average age of the non-syndromic cases was 63. There was significantly less overall medial degeneration in subjects with BAV (n=31) than in the other syndromic cases ($p=2.2x10^{-3}$). Among the non-syndromic cases, overall medial degeneration increased with age. This was the result of increasing amounts of smooth muscle cell nuclear loss, laminar medial collapse and mucoid extracellular matrix accumulation (MEMA).

Conclusions: The new AECVP/SCVP Aorta Consensus Grading Scheme is a robust method for general surgical pathology signout and research studies. New, less common terminology will require increasing familiarity for consistent implementation. A new uniformity between centers can now be accomplished. Even within a small study set, we uncovered significant differences associated with syndromes and aging. With increasing use and larger study sets, it may now be possible to associate histopathologic findings with specific syndromes and increase the value of the surgical thoracic aorta.

Cytopathology

341 Can the Ki-67 Index Evaluated on Fine Needle Aspiration Cellblock Material Reliably Grade Pancreatic Neuroendocrine Tumors?

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Background: Currently, the best predictor of pancreatic neuroendocrine tumor (PNET) behavior is the tumor grade determined by measuring mitotic activity or/and Ki-67 index on surgically resected specimens. Although fine needle aspiration (FNA) is widely used in the preoperative diagnosis of PNETs, its role in prognostic evaluation of the tumors is very limited. The goal of this study was to assess the tumor grade, as determined by the Ki-67 index, on FNA cellblock material and correlate with the final grade on resection specimens.

Design: The institutional pathology database was searched for PNETs diagnosed by FNA between January 2006 and August 2015. Only the cases with available cellblock material and corresponding surgical specimens were included in the study. Clinicopathologic characteristics, cytological and surgical follow-up cases were retrospectively reviewed. The cytological diagnosis of PNETs was rendered by cytomorphologic analysis and adjunctive chromogranin and synaptophysin immunostains. Surgically resected tumors were graded according to 2010 WHO classification and staged following 2010 AJCC staging criteria. Ki-67 immunostain was retrospectively performed on FNA cellblocks and resected tumors if not performed initially.

Results: A total of 48 FNA cases with corresponding resections were identified. The cellblock sections contained >1000, 500-1000, 250-500, 100-250, and <100 tumor cells in 23, 9, 10, 2 and 4 cases, respectively. The resected tumors ranged from 0.9 to 8.5 cm (mean = 3.2 cm) and were graded as G1 and G2 tumors in 24 and 24 cases, respectively. In G1 tumors, the Ki-67 grading based on cellblock sections showed a 100% correlation rate with the grading on surgical specimens, regardless of cellblock cellularity and tumor size. However, in G2 tumors the correlation rate between cellblocks and surgical

specimens was only 33% (8 of 24 cases). Furthermore, the correlation rate decreased with lower cellblock cellularity, with the rates being 55% (6/11) in cases with >1000 cells, 40% (2/5) in cases with 500-1000 cells and 0% (0/8) in cases with <500 cells. The decrease in correlation rates in G2 tumors was also independent of tumor size.
Conclusions: The Ki-67 index may be significantly underestimated on FNA cellblock material, which can result in tumors with a final G2 grade being erroneously categorized as a G1 tumor by FNA. This is more likely to occur in cases with low cellularity. Our results suggest that caution should be advised in utilizing and interpreting the Ki-67 index as a prognostic marker/tumor grading on FNA specimens.

342 The Value of Negative Diagnosis in Thyroid Fine-Needle Aspiration: A Retrospective Study with Histologic Follow-Up

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Background: The Bethesda System for reporting thyroid cytopathology (BSRTC) predicts an incidence of malignancy of less than 5% in cases with a benign diagnosis on fine needle aspiration (FNA). However, recent series have suggested that the true rate of malignancy might be significantly higher in this category of patients. The aim of this study was to review our experience at a large academic center by evaluating the risk of malignancy in these patients with negative thyroid FNAs.
Design: We performed a retrospective analysis of patients with benign thyroid FNA results who underwent thyroidectomy (2008 to 2013). Information including demographics, ultrasound features, FNA diagnosis and surgical follow-up information were recorded. Slides were reviewed on cytology-histology discrepant cases and it was determined whether the discrepancy was due to sampling or interpretation error.
Results: A total of 822 FNA cases with a benign diagnosis from 718 patients with surgical follow-up were identified. The patients included 597 females and 121 males, aged 12 to 89 years (mean=51). Original FNA diagnoses included 761 cases of benign goiter (including cyst contents and colloid nodules) and 61 cases of lymphocytic thyroiditis. On subsequent surgical resection, 160 cases were found to be neoplastic (35 benign neoplasms [follicular and Hürthle cell adenoma]; and 125 (15%) malignant neoplasms [116 papillary thyroid carcinoma (PTC), 4 follicular carcinoma, 2 medullary thyroid carcinoma, 2 Hürthle cell carcinoma, and 1 lymphoma]). The remaining 662 cases were confirmed as benign and non-neoplastic. Retrospective review of the false negative cases showed that 90% were due to sampling error and 10% were due to interpretation error. Interpretation error was more likely to occur in the follicular variant of PTC (44% of cases missed due to interpretation error), follicular carcinoma (20% of cases missed due to interpretation error); and background lymphocytic thyroiditis. Tumor size was larger in cases with interpretation error (mean=2.5) than in cases with sampling error (mean=0.82). The most common reasons for surgical intervention in benign thyroid FNA were suspicious or malignant diagnosis in a separate nodule, large size and symptomatic nodules
Conclusions: The false-negative rate of thyroid FNA is higher than suggested by the BSRTC. False negative FNA diagnoses were due to sampling error in the overwhelming majority of cases. Due to this inherent limitation of cytology and the diagnostic criteria/ subtlety of certain entities, a negative FNA does not exclude cancer with certainty.

343 Lymphoma Diagnosis on Endoscopic Ultrasound Guided Fine Needle Aspiration Specimens of Abdominal Lymphadenopathy; a Retrospective Study at an Academic Tertiary Referral Center

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Background: Endoscopic Ultrasound guided Fine Needle Aspiration (EUS-FNA) is indicated in suspected diagnosis of lymphoma. EUS-FNA allows sampling of abdominal lymphadenopathy (celiac axis, peri-gastric, peri-pancreatic, peri-portal) easily and safely with adequate tissue for diagnosis. Lymphoma represents less than 5% of EUS-FNA diagnosis on abdominal lymphadenopathy/mass. We evaluated EUS-FNA specimens with lymphoma of deep-seated lymph nodes.
Design: All EUS-FNA specimens with non-Hodgkin lymphomas (NHL) and Hodgkin lymphoma (HL) diagnosis performed (2003-2015), were retrieved. Rapid on site evaluation was done in almost all cases. Ancillary studies (Immunohistochemistry, PCR, FISH) on available cell block and flow cytometry (FC) were performed when possible. Clinicopathological data were collected.
Results: A total 58 cases of lymphoma were identified, including 55 NHL and 3HL. EUS-FNA specimens were diagnostic for lymphoma in 51 cases and suspicious for lymphoma in 7 cases (6 suspicious for NHL). In 89% of specimens (49/55 cases) the diagnosis was definitive for NHL. In 24 cases, there was a tissue biopsy (lymph node, liver, gastric) that confirmed the EUS-FNA findings. NHL cases were subclassified as: 18 Follicular lymphoma (FL), 21 Large B-cell lymphoma (LBCL), 3 small lymphocytic lymphoma (SLL), 3 extra nodal marginal zone B-cell lymphoma (MZL), 1 anaplastic large cell lymphoma and 1 Burkitt lymphoma. Subclassification was not possible in 3 cases (where cytology was NHL with 1 clonal, 1 biclonal, and 1 aberrant B cell population identified by FC). The cases suspected for NHL included 1 FL, 3 LBCL, 2 low grade NHL (MZL, FL, others,); for these cases, no material was available for analysis by PCR or FC. There was 1 case of Lymphocyte-depleted Hodgkin's lymphoma in the liver, confirmed on biopsy, 1 recurrent HL and 1 suspected for HL.
Conclusions: EUS-FNA allows adequate material for the rapid diagnosis of lymphoma in the intra abdominal location. When cytomorphologic studies were used in combination with ancillary studies (Immunohistochemistry, flow cytometry, PCR, FISH), we were able to provide diagnosis of lymphoma on EUS-FNA samples from deep-seated lymph nodes/ masses. EUS-FNA is accurate for diagnosing and typing common forms of lymphoma specially LBCL, FL and low grade NHL (MZL, SLL). Our study supports the use of EUS-FNA specimens in cases suspected of lymphoma.

344 Hodgkin Lymphoma Diagnosed by Fine Needle Aspiration Cytology: A Retrospective Review of 110 Consecutive Cases with Histopathologic Correlation

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Background: The diagnosis of Hodgkin lymphoma (HL) by FNA requires the identification of Reed-Sternberg cells in an appropriate inflammatory milieu. Historically, the diagnostic accuracy of fine needle aspiration (FNA) for HL has been high. However, the use of FNA for the primary diagnosis of lymphoma remains controversial and data specifically analyzing the role of FNA in the diagnosis of HL is limited.
Design: A computerized search of our laboratory information system was performed for 1990-2015 to identify all FNA cases diagnosed as HL, suspicious for HL, or atypical cells cannot exclude HL. Correlating tissue biopsies, when performed, were also reviewed and those histopathologic results were compared with the original FNA diagnoses. Diagnostic discordance with the tissue diagnosis was graded as minor (sampling or subtyping issues) and major (false positive; a change from at least suspicious for malignancy to a benign diagnosis).
Results: 88 (85%), 15 (15%), and 7 (6%) cases were diagnosed by FNA as HL; suspicious for HL; and atypical cells, cannot exclude HL, respectively. 53 (60%) of the positive FNAs had corresponding core biopsy results with confirmation of HL in 47 (89%). 11 (73%) of the suspicious FNAs had correlating core biopsies with confirmation of HL in 8 (73%). There were no false positive FNAs and all correlating positive tissue biopsies were diagnosed as HL. 6 (86%) of the FNAs diagnosed as atypical had correlating tissue results with confirmation of HL in 3 (50%).
Minor diagnostic discordance between FNA and histopathologic results was noted in 12 (17%) cases. In 8 cases, the FNA diagnosis was positive for HL while the tissue diagnosis was indeterminate or negative due to suboptimal tumor volume in the surgical biopsy. There was 1 case diagnosed by FNA as HL, favor mixed cellularity type that was diagnosed as classical HL, nodular sclerosis type on biopsy. In 3 cases diagnosed as atypical by FNA, 2 were diagnosed as reactive hyperplasia on core biopsy and 1 was considered nondiagnostic due to suboptimal tissue.
24 (22%) of the positive FNA cases were diagnosed in the setting of disease recurrence.
Conclusions: FNA is a reliable means of diagnosing HL, particularly for documenting disease recurrence, although subclassification is better assessed by tissue biopsy. Concordance between FNA and histopathologic diagnosis is high when adequate tissue is collected. In a subset of cases, FNA material was superior diagnostically when compared to the corresponding surgical biopsy.

345 The Development of a Telomerase Immunocytochemical Assay for the Detection of Urothelial Neoplasms in Urinary Tract Cytopathology Specimens

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Background: While the positive predictive value (PPV) of urinary tract (UT) cytopathology is excellent for high grade urothelial carcinoma (HGUC), it has relatively poor sensitivity for HGUC and low grade urothelial carcinoma (LGUC) and poor PPV for LGUC. While several ancillary tests have been developed to improve its diagnostic quality, none have gained universal acceptance. Urothelial neoplasms upregulate the expression of the telomerase enzyme, which lengthens chromosomal telomeres and is required for cell immortalization and tumor growth. Using an immunocytochemical (ICC) technique, we studied the expression of telomerase in a small cohort of UT specimens to determine whether this method could improve the detection of UT neoplasms.
Design: 56 fresh voided urine specimens were split and cytospins were created. Specimens were stained in the immunopathology laboratory along with control slides using a telomerase-specific antibody (ab) (Anti-hTERT ab, SCD-A7) developed and provided by Sienna Cancer Diagnostics Ltd. The slides were interpreted blindly by a cytotechnologist [CT] and cytopathologist [CP] and scored as positive, negative, inadequate, or equivocal.
Results: The telomerase assay demonstrated improved PPV (71% [CT], 50% [CP]) over traditional cytomorphology (~30%) for LGUC and HGUC while maintaining excellent specificity (94% [CT], 72% [CP]). Equivocal diagnoses were often secondary to heavy background debris or degenerative cell staining, but this background staining occurred predominantly in specimens belonging to patients who had urothelial malignancies on follow up. When the assay was combined with "atypical" UT specimens, the PPV was 100% [CT] and 83% [CP].

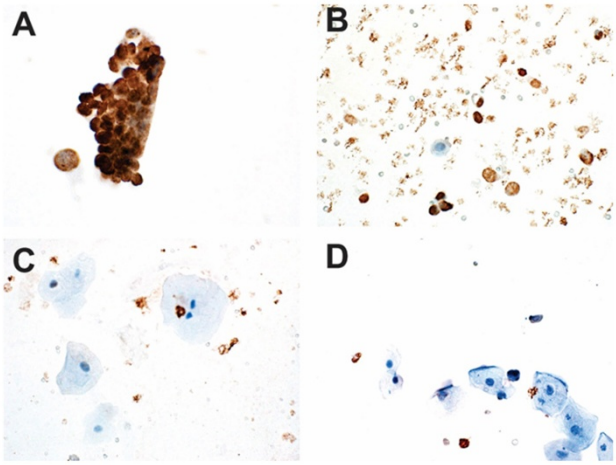

Figure 1. A, High grade urothelial carcinoma (HGUC) present as a single cell and fragment in this field, demonstrating strong positive nuclear staining for telomerase. B, Several single HGUC cells staining strongly positive for telomerase with a benign urothelial cell in the center and bacterial colonies in the background (positive by telomerase as well). C, Negative staining in benign cells with bacterial colonies and debris in the background. D, Negative staining in benign cells.

Conclusions: The use of telomerase ICC shows promise as an ancillary test for the detection of urothelial neoplasms in UT specimens. This assay is capable of detecting both HGUC and LGUC when present in urine. Malignant specimens but not benign specimens may demonstrate heavy background debris or degenerative cell staining, which suggests immediate specimen processing and additional washing steps during preparation could improve the diagnostic quality of this assay.

346 Comparing Outcomes of hrHPV Positive Pap Tests: HPV 16/18 Genotypes Versus Non-16/18 Genotypes

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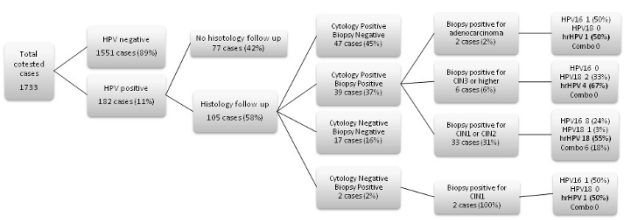
Background: Recent literature has discussed histologic outcomes in women with negative cytology and positive hrHPV test results and has established the increased risk these women have for cervical lesions. The most common high risk types, HPV-16 and 18, are the best documented; however, the contribution of non-HPV16/18 genotypes to cervical lesions is less well known. We compared the outcomes of non-16/18 hrHPV genotype positive cases to outcomes for HPV-16 or 18 genotypes to explore the histologic follow up differences and the respective positive predictive values of these two categories.

Design: Co-testing results with HPV genotyping using the Cobas® 4800 HPV test at our institution were evaluated over a period of 15 months. The data was tabulated and categorized into HPV negative and HPV positive categories. For the HPV positive arm, cases were correlated with cytology results as well as subdivided into three genotype categories: HPV-16, HPV-18 and other hrHPV (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). Follow-up cervical biopsy results and calculated positive predictive values were used to compare the different groups.

Results: Co-testing with HPV genotyping was performed on 1733 samples. Of these, the HPV DNA result was negative for 1551 cases (89%) and positive for 182 cases (11%). Histological follow-up was available on 105 (58%) of the HPV positive cases. Of these, 64 cases (61%) had negative histology and 41 cases (39%) were lesional. The study design and genotyping of the 41 lesional cases are outlined in Figure 1. Of note, five non-16/18 hrHPV positive cases were biopsy proven CIN 3 or higher. The calculated positive predictive value of a non-16/18 hrHPV positive case resulting in a biopsy proven lesion was 33%, compared to 45% for HPV 16 and 50% for HPV 18.



Conclusions: Our early experience suggests the positive predictive value of women with a non-16/18 hrHPV positive result having a cervical lesion is relatively high (33%), irrespective of the cytology diagnosis. Although this predictive value is less than that for the 16 and 18 genotypes (45% and 50%, respectively), it is a stronger correlation than anticipated. Additional studies are necessary to determine the impact of this finding across a larger population.

347 Molecular Adequacy of Fine-Needle Aspiration Cytologic Smears for Next Generation Sequencing in Lung Adenocarcinoma

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Background: Multi-gene testing on cytologic smears offers many advantages to formalin-fixed tissue such as the ability to perform onsite evaluation of "molecular adequacy" and the acquisition of higher quality DNA. Our study aims to assess the adequacy of FNA smears for interrogation of multiple gene variants using next generation sequencing (NGS).

Design: 15 cases of lung adenocarcinoma with air-dried Diff-Quik smears for which there was NGS performed on the formalin-fixed cell block (N=4) or surgical resection specimen and cell block (N=11) were used. Neoplastic cellularity for all specimen types was documented. Qubit flurometric quantitation was performed and the DNA was subjected to a targeted NGS assay that interrogates 2800 variants in 50 cancer related genes.

Results: 15 patients with a diagnosis of lung adenocarcinoma (N=14) and lung adenosquamous carcinoma (N=1), were included in the study. Immunohistochemistry for TTF-1 and napsin A was positive in 94% of the cases. DNA concentration of the smears ranged from 2.8–81.2 ng/mL and the neoplastic cellularity ranged from <1%-80%. 14 of 15 FNA smears were successfully sequenced. The case that failed was an adenosquamous carcinoma with <1% tumor cellularity on the smear. 85% of the mapped reads were on target. Targetable genetic alterations (Table 1) were identified in 9 of 14 smears (64%). There was 86% concordance between FNA smears, cell block and surgical tissue. One discordant case harbored a variant in the surgical specimen. This patient had numerous nodules that may have contributed to tumor heterogeneity in the specimen. The other discordant specimen identified an additional *EGFR* T790M mutation in the smear but not the cell block.

Conclusions: Cytology smears provide reliable results for identification of actionable variants in lung adenocarcinoma. Analyses that characterize molecular adequacy may help to further qualify this specimen type.

	FNA Smear N=15	FNA Cell Block N=15	Surgical Resection N=11
Average DNA concentration (ng/uL)	11.4	5.5	58.8
Average neoplastic cellularity (%)	48	31	68
Average coverage	1580X	1350X	1140X
Actionable variants detected (=#)	*EGFR* exon 19 del=2 *EGFR* G719=2 *EGFR* L858R=1 ***EGFR* T790M=1** *KRAS* G12C=4 *KRAS G13D*=1 *PIK3CA* T1025I=2	*EGFR* exon 19 del=2 *EGFR* G719=2 *EGFR* L858R=1 *KRAS* G12C=4 *KRAS* G13D=1 *PIK3CA* T1025I=2	*EGFR* exon 19 del=2 *EGFR* G719=2 *EGFR* L858R=1 *KRAS* G12C=4 *KRAS* G13D=1 ***KRAS* Q61H=1** PIK3CA T1025I=2
Other variants detected	*TP53, APC, STK11, CTNNB*	*TP53, APC, STK11, CTNNB*	*TP53, APC, STK11, CTNNB*

348 Utility of GATA3 in Urothelial Cytology

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Background: GATA binding protein number 3 (GATA3) is a zinc finger transcription factor which plays a role in directing cell proliferation and differentiation. Heavily researched in recent years as a sensitive marker for malignancies of both breast and urothelial origin, GATA3 is reportedly constitutively expressed in normal urothelium with upregulation in the setting of high grade and invasive tumors. To our knowledge, there have been no published studies which examined GATA3 staining pattern variations in urothelial cytology specimens. Our aim was to examine whether the reported upregulation of this transcription factor in malignancy would be helpful in identifying urothelial carcinoma in cytological specimens.

Design: Urothelial cytology specimens (bladder barbotages, ureteric washes, pelvic brushes and voided urines) were processed according to standard departmental protocol. Following the creation of Thin Prep specimens for pap staining and cytologic diagnosis, residual material from the vials was used to create an additional Thin Prep slide for each case. These additional preparations were then stained with anti-GATA3 monoclonal antibody (Roche, L50-823 – mouse). GATA3 nuclear staining was blindly evaluated and scored on a three tiered scale (negative, weak, strong). The GATA3 staining results were then correlated with the cytologic diagnoses rendered using the Paris classification system for urine cytology.

Results: Results from a total of 45 specimens from 44 patients demonstrated strong nuclear staining for GATA3 exclusively in samples that were deemed abnormal cytologically (atypical to malignant). GATA3 staining was negative to weak in all cases which were diagnosed as negative for urothelial carcinoma on cytology. In this study, the specificity of strong GATA3 staining in urothelial cytology specimens was 100%.

	Abnormal Cytology	Negative Cytology
GATA3 strong positive	5	0
GATA3 negative to weak	5	34
	Sensitivity - 50%	Specificity- 100%

Conclusions: This preliminary study suggests that GATA3 appears to indeed be upregulated in urothelial lesions and malignancies and that this upregulation may

be manifested as differential staining patterns in negative versus abnormal urothelial cytologies. With a high degree of specificity for lesional cells, GATA3 may prove to be a useful diagnostic aid in urothelial cytology.

349 ATRX and DAXX Immunohistochemistry (IHC) in Pancreatic Neuroendocrine Tumor (PNET) Fine Needle Aspirations (FNA)- Can They Be Clinically Useful?

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Background: Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX) gene and Death domain-associated protein gene (DAXX) encode proteins in chromatin remodeling and have been described in PNET. Mutations in these proteins (resulting in loss of normal nuclear localization) leads to tumor progression by alternative lengthening of telomeres (ALT). Furthermore, a loss of DAXX and/or ATRX has been shown to be associated with poor prognosis. Recently, ATR inhibitors have been described which induce cell death in cancer cells that rely on ALT. Hence, ATRX and DAXX IHC may play a role in selecting appropriate targeted therapy. PNETs are frequently diagnosed on FNAs and ATRX and DAXX IHC in FNAs have not been evaluated, before our current study.

Design: We selected 24 FNA cell blocks (CB) of PNET. ATRX (Sigma-Aldrich,1:100), DAXX(Sigma-Aldrich,1:200), Synaptophysin (Biogenix,CA,1:640), Chromogranin (Leica,IL,RTU), Ki-67/MIB1 (DAKO,1:80) staining was performed. Nuclear staining was graded by intensity (0-3+) and percent of positive tumor cells and a combined score of >5 was considered positive.

Results: All 24 cases of PNET were positive for synaptophysin and chromogranin. The tumors were classified according to the WHO 2010 system.

Table 1. IHC Results

	Number	ATRX+(%)	ATRX-(%)	DAXX+(%)	DAXX-(%)
MIB1<3%	15	11(73)	4(27)	1(7)	14(93)
MIB1 3-20%	5	3(60)	2(40)	3(60)	2(40)
MIB1>20%	4	3(75)	1(25%)	3(75)	1(25)

Table 2. Tumor grade and IHC Results

	ATRX+/DAXX+(%)	Loss of ATRX/DAXX/both(%)
Low grade	3(15)	17(85)
High grade	2(50)	2(50)

Conclusions: DAXX is a weak-staining antibody and hence a combined score of intensity and percent tumor staining is more useful in evaluation of expression. Loss of DAXX (80%) is more common than loss of ATRX (30%) in low-grade PNETs, suggesting that DAXX is probably lost first in the chromatin remodeling pathway. Loss of ATRX and/or DAXX is more common in low-grade PNETs than high-grade PNETs, which underscores the fact that low-grade PNETs are clinically and genetically distinct from high-grade PNETs. Our results are similar to those published for pancreatic surgical resections. We conclude that in an era of personalized medicine, FNAs can reliably be used to analyze ATRX and DAXX IHC expression and hence may play a role in evaluation for appropriate targeted therapy, especially in low-grade PNET.

350 PLAG1: An Immunohistochemical (IHC) Marker with Limited Utility in Separating Pleomorphic Adenoma (PA) from Other Basaloid Salivary Gland Tumors

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Background: Fine needle aspiration (FNA) diagnosis of salivary gland neoplasms is often challenging. Differentiating between PA and other basaloid neoplasms especially basal cell adenoma (BCA) and adenoid cystic carcinoma (AdCC) can be difficult in cellular aspirates. PLAG1 (pleomorphic adenoma gene1) is a protocooncogene which is frequently rearranged in PAs, leading to the aberrant expression of its protein. PLAG1 IHC expression has been reported to be positive in most PAs. The aim of the study is to evaluate the sensitivity and specificity of PLAG1, to differentiate PA from other basaloid neoplasms.

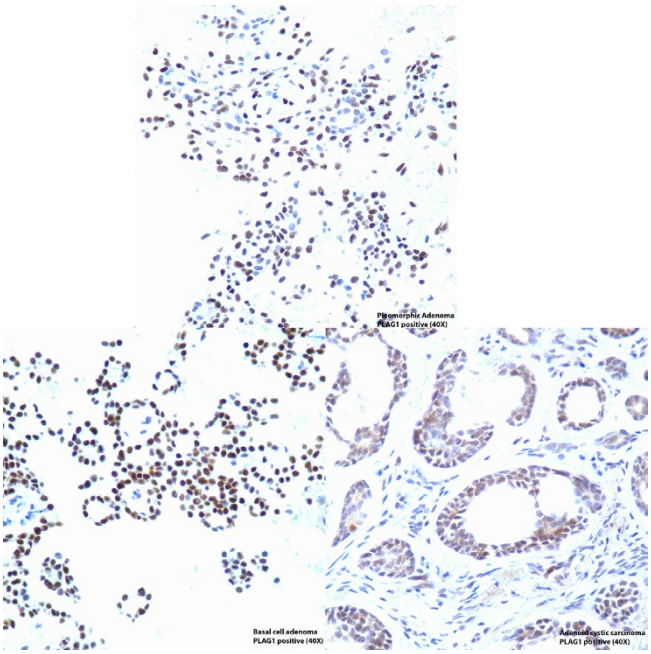
Design: We examined 125 cases, including 52 FNA's (40 PA, 5 BCA, 7 AdCC) and 73 surgical excisions (SE) (36 PA, 8 BCA, 29 AdCC). IHC staining for PLAG1 (Clone 3B7, Sigma-Aldrich, 1:20), was performed on FNA cell blocks and SE blocks. Nuclear staining of tumor cells was scored by intensity (0-3) and percentage of tumor cells. A combined score (intensityx% tumor cells) of >5 was defined as positive.

Results: Table1: IHC staining results of PLAG1

	No. of cases		PLAG1 Positive	
	FNA	SE	FNA(%)	SE(%)
PA	40	36	22(55)	33(91.6)
BCA	5	8	3(60)	5(62.5)
AdCC	7	29	0(0)	12(41.3)
Total	52	73	25	50

Table2: Statistical analysis of PLAG1 for PA

	FNA(%)	SE(%)	FNA&SE(%)
Sensitivity	55	92	72
Specificity	75	54	59
Positive Predictive Value	88	66	73
Accuracy	60	73	67



Conclusions: In FNAs, the sensitivity (55%) and specificity (75%) of PLAG1 are relatively modest thus limiting its diagnostic utility. However, the sensitivity (92%) of PLAG1 in supporting a diagnosis of PA in SE is relatively high but it is not specific. BCA and AdCC also showed PLAG1 false positivity in SE, but less so in FNA, with virtually no positivity expressed in AdCC. This may be due to limited sampling of these tumors on FNAs, as well as tumor heterogeneity. This should be recognized when assessing samples with limited cellularity. Hence, our results show that PLAG1 cannot be used as a single stand alone IHC marker for PA to reliably distinguish it from other basaloid tumors especially in FNA specimen.

351 Cytologic Predictors of Malignancy in Bile Duct Brushings (BDBs): A Multi-Reviewer Analysis of 60 Cases

Vaidehi Avadhani, Ezgi Hacihasanoglu, Krisztina Z Hanley, Uma Krishnamurti, Alyssa M Krasinskas, Adeboye O Osunkoya, Lauren Daniels, Alexa Freedman, Michael Goodman, Volkan Adsay, Michelle Reid. Emory University, Atlanta, GA.

Background: Evaluation of BDBs is challenging and has a purported low sensitivity for detection of malignancy.

Design: 7 reviewers (with variable experience in cytology) blindly reviewed 60 BDBs, [30 malignant, by histologic confirmation and 30 benign(15 with stent), with either resection/≥18 mo uneventful f/u] and diagnosed (Dx) them as benign, atypical, suspicious or malignant using 14 "malignant" cytologic criteria that were based on agreement between ≥2/3 cytologists(Table1).

Results:

Table 1

Criteria	Prevalence %		p value	OR	95%CI
	Malignant n=30	Benign n=30			
Hypercellularity	23	0	0.005	-	-
3D groups	52	3	<0.0001	31.1	3.7-259
Cellular Discohesion	38		0.001	17.7	2.1-149
High N/C (>50%)	48		<0.0001	27.1	3.2-225
Nuclear molding	20	0	0.01	-	-
Cytoplasmic vacuoles	43	13		4.9	1.4-17.8
Inflammation		33	0.42	1.5	0.5-4.4
2-Cell population	57	0	<0.0001	-	-
Hypo/Hyperchromasia	70	10		21.0	5.1-87.4
Nuclear irregularity	67	13		13.0	3.6-47.9
Prominent nucleoli	21	0	0.008	-	-
Pleomorphism	62	3	<0.0001	47.5	5.6-399
Necrosis	17	13	0.71	1.3	0.3-5.4
Mitoses	0		-		

11/14 criteria were significantly associated with malignancy. Cytoplasmic mucin vacuoles and necrosis were equally identified in benign and malignant BDBs including stented and non-stented BDBs.

Table 2.

Score No. of criteria present (of 14)	Malignant n(%)	Benign n(%)	p-value
0	4 (20)	16(80)	0.0002
1	2(18)	9 (81)	0.003
2	3(75)	1(25)	0.16
3	2(40)	3(60)	0.53
4	1(100)	0	-
5	1(100)		
6	1(100)		
7	4(100)		
8	5(100)		
9	2(67)	1(33)	0.41
10	4(100)	0	-
11	1(100)		

53% of BDBs with a benign outcome had none of the 14 criteria (Table2). Of the 20 BDBs with ≥ 4 criteria, only 1(5%) had a benign outcome. Score cut point ≤3 compared to ≥4 yielded the best sensitivity (63%), specificity (97%) and accuracy (80%) for malignancy. For every unit increase in score in malignant criteria the odds of malignancy were 1.82X that of benign.

Conclusions: 3D clusters, high N/C, irregular nuclei, hypercellularity, discohesion, hyper/hypochromasia and 2-cell populations were used most frequently by reviewers who rendered an accurate malignant diagnosis. Presence of ≥4 criteria helps to objectively identify malignancy in BDBs.

352 Do HPV Types and Infection Patterns Affect the Cytologic Detection of High Grade Cervical Lesions on Pap Test?

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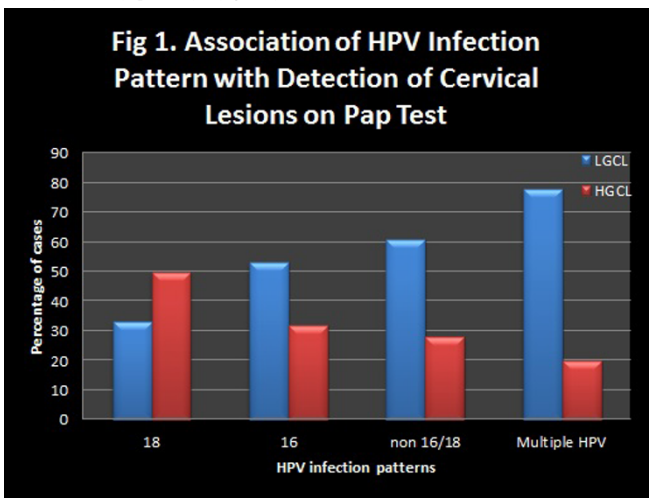
Background: Persistent infection with high-risk HPV(hrHPV) is the major cause of cervical precancerous lesions. The effect of HPV infection pattern on cytologic detection of significant lesions is unclear. This study aimed to determine the association between HPV infection patterns and Pap test sensitivity in women with HSIL.

Design: 257 biopsy-proven HSIL cases with prior HPV testing were identified from 171,621 Pap tests performed from March 2013 - June 2014. The cytology and biopsy interpretations were rendered by board-certified cyto- or gynecologic pathologists in an academic medical center. Cytologic evaluation and hrHPV testing were performed on ThinPrep (TP, n=133) and SurePath (SP, n=124) samples.

Results: In 257 biopsy-proven HSIL cases, preceding Pap tests within 14 months showed NILM in 20 (8%), low-grade cervical lesions (LGCL, including ASCUS and LSIL) in 166 (64%), and high-grade cervical lesions (HGCL, including ASC-H, AGC and HSIL) in 71 (28%). TP and SP platforms showed similar sensitivity in detecting squamous cell abnormalities (92% and 94%). However, SP demonstrated significantly higher sensitivity in detecting HSIL (23/124) than TP (11/133) ($p<0.02$). hrHPV was positive in 161 of 184 tested cases with a sensitivity of 87%. LGCLs detected on Pap test were often associated with infections of multiple HPVs (31/40, 78%) and non-16/18 hrHPV (33/54, 61%) compared to single genotype infections of HPV 16 (33/62, 53%) or 18 (2/6, 33%) ($p<0.05$) - (figure1).



In contrast, HGCLs on cytology were seen more often in single genotype infections of HPV18 (3/6, 50%) and 16 (20/62, 32%), compared to non-16/18 (15/54, 28%) and multi-genotypic infection (8/40, 20%) (p=0.219). Of note, 55% (12/22) of the HPV-HSIL were detected by Pap test.

Conclusions: LGCL cytomorphology was significantly more often associated with multiple HPV and non-16/18 hrHPV infections. In contrast, the HGCL cytomorphology was more often seen with single genotype infections of HPV16/18 though this was not statistically significant, likely due to cohort size. Our findings suggest that non-16/18 or multiple HPV infections produce predominantly lower-grade cytomorphology or smaller lesions than HPV 16/18, which may result in Pap test underdiagnosis.

353 Suspicious AFIRMA® Gene Expression Classifier Test Result in Thyroid Fine Needle Aspiration Cytology: Implications for Patient Management

Rebecca Baldassarri, Susan Fernandez, Angelique W Levi, Malini Harigopal, Diane Kowalski, Guoping Cai, Kevin Schofield, Adebowale Adeniran. Yale University School of Medicine, New Haven, CT.

Background: Up to 30% of thyroid nodules are classified as indeterminate by fine needle aspiration (FNA). This category includes follicular lesion of undetermined significance (FLUS), follicular neoplasm (FN), and suspicious for malignancy (SFM). Only 5-15% of these indeterminate thyroid nodules are found to be malignant after resection, prompting the need for better preoperative characterization of indeterminate nodules in order to reduce unnecessary surgery. The AFIRMA® gene expression classifier (GEC) test is a RNA based, microarray approach used for this purpose. The goal of our study was to evaluate our institutional experience with AFIRMA® GEC analysis for thyroid nodules with indeterminate diagnosis on FNA.

Design: Eighty-seven cases of thyroid FNA with indeterminate diagnosis and concomitant AFIRMA® testing were selected from our files over a 3-year period (2012-2015). Information including demographics, ultrasound features, FNA diagnosis, AFIRMA® results and surgical follow-up information (when available) were recorded.

Results: A total of 86 cases from 83 patients were reviewed. This included 68 female and 15 male patients (age range 18-86 years). The average size of nodule was 2.5 cm (range 0.6-6.9). Cytologic diagnoses were FLUS in 65 (76%), FN in 10 (12%), Hürthle cell neoplasm in 9 (10%) and SFM in 2 (2%) cases. AFIRMA® results were benign in 10, suspicious in 73 cases and unsatisfactory in 3 cases. Surgical follow-up was available in 67 cases: 61 (91%) cases reported as suspicious, 4 (6%) as benign and 2 (3%) as insufficient by AFIRMA®. Of the cases with suspicious AFIRMA® results and surgical follow-up (61 cases), 17 (28%) were malignant (classic PTC – 12, Follicular variant of PTC – 4, follicular carcinoma – 1, Hürthle cell carcinoma – 2, medullary carcinoma – 1) and 44 (72%) were benign. All 4 cases with benign AFIRMA® result were benign on surgical follow-up.

AFIRMA® MOLECULAR DIAGNOSIS	SURGICAL DIAGNOSIS MALIGNANT	SURGICAL DIAGNOSIS BENIGN
SUSPICIOUS	17	44
BENIGN	0	4
TOTAL	17	48

Conclusions: With a malignancy rate of 28% in cases with suspicious AFIRMA® result, a suspicious AFIRMA® result is of limited value to surgical candidates in a large surgical center such as ours. While our results demonstrate a very high negative predictive value of the AFIRMA® test, and thus, its potential as a triaging tool, particularly in non-surgical patients, this use is limited by the small number of cases with a benign AFIRMA® result and benign surgical resection in this study.

354 Experience with UroVysion FISH in Suspicious Cases for Urothelial Malignancy from a Large Academic Medical Center

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Background: Bladder cancer is the fourth most common cancer in men and the fifth most common cancer worldwide. UroVysion fluorescence in situ hybridization (FISH) can detect urothelial carcinoma with high sensitivity and specificity. This assay is commonly used for detecting aneuploidy for chromosomes 3, 7, 17, and deletion of 9p21 in exfoliated urothelial cells in the bladder. Polysomies of chromosome 3, 7 and 17 correlate mostly with high grade bladder cancer whereas deletion of 9p21 is frequently associated with low grade urothelial carcinoma. Hence, this assay may help detection and prognostication of urothelial carcinoma in cytologically suspicious cases.

Design: A total of 1039 patients with atypical urinary cytology or bladder biopsies suspicious for urothelial neoplasm were evaluated by UroVysion FISH over a 12 year period at the University of Pittsburgh. Voided and instrumented urines from the bladder, renal pelvis, and ureters were used to prepare Thin Prep slides. Multi-color FISH was performed using the UroVysion Kit for CEP3, CEP7, CEP17 and LSI p16 labeled in SpectrumRed, SpectrumGreen, SpectrumAqua and SpectrumGold (Abbott Molecular), respectively. Analysis was performed using a BioView Duet Automated Imaging and Analysis system (BioView Inc., Billerica, MA). UroVysion FISH Interpretations were classified as positive, negative, unsatisfactory, or inconclusive. A minimum of 25 cells were analyzed using established FDA criteria A specimen was considered positive if 4 or more cells had gains of multiple chromosomes indicated by >2 signals for two or more probes and/or exhibited homozygous deletion of 9p21 in 12 or more cells. An adequate sample not meeting the above criteria was considered negative. A specimen with less than 25 analyzable cells was deemed unsatisfactory for evaluation.

Results: 237 cases were positive by UroVysion FISH [trisomy (n=182), 9p21 deletion (n=45), both 9p21 deletion and trisomy (n=10)]. 93 cases were unsatisfactory due to insufficient cells, degenerated cells and / or obscuring blood and squamous cells. 37 cases were equivocal/ inconclusive. All remaining 672 cases were negative. An average of 155 cells was analyzed per case.

Conclusions: UroVysion FISH increases the sensitivity and specificity of urine cytology and cystoscopy for the detection of urothelial neoplasia, and provides additional cytogenetic information to support an equivocal morphologic diagnosis. Histologic correlation and clinical follow-up are strongly advocated.

355 Utility of Fine Needle Aspiration and Core Needle Biopsy to Pre-Operatively Assess Renal Lesions

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Background: Image-guided biopsy is often performed to evaluate solid renal masses. The need for such biopsies has increased owing to the growing number of small, incidentally detected renal lesions. Fine needle aspirates (FNA) and core needle biopsies (CNB) with touch preparation may be performed separately or together to assess renal masses. Literature comparing FNA and CNB alone or in combination in this setting is limited. The aim of this study was to evaluate the diagnostic yield of renal FNA and/or CNB at our institution.

Design: Archival renal lesions evaluated by FNA and/or CNB with touch preparation from 2012 to 2015 were collected. Clinical data (age, gender), lesion size, biopsy details (procedure type, number of passes, tissue volume), specimen adequacy, diagnoses, complications, and histologic follow up were recorded.

Results: A total of 51 cases were identified, where 30 had both FNA and CNB, 15 only FNA, and 6 cases only CNB with touch prep. The table compares clinical findings, adequacy and final diagnoses findings for each procedure group.

Biopsy precedue	FNA only (N=45)	CNB alone (N=36)	FNA with CNB (N=30)
Patient age (years)	69.4	67.4	66.7
Average renal lesion size (cm)	4.7	4.1	4.3
Sex (M:F)	28:17	19:17	17:13
Number of passes	3.0	2.1	4.8
On-site specimen adequacy	89%	88%	100%
Final specimen adequacy	95%	89%	93%
Non-diagnostic cases	4%	11%	7%
Cases with negative diagnoses	9%	11%	10%
Atypical/suspicious diagnoses	22%	8%	10%
Positive for neoplasm/ malignancy	64%	69%	73%

Renal epithelial lesions were the most common diagnoses (27 cases, 53%). The remaining lesions with a definitive diagnosis included urothelial carcinoma (4 cases, 8%), metastases (5 cases, 4%), hematolymphoid neoplasms (2 cases, 4%), and angiomyolipoma (2 cases,4%) Bleeding complications noted while on-site occurred in 3 (7%) FNA cases and 1 (3%) CNB.

Conclusions: Image-guided FNA and/or CNB was useful in managing patients with renal masses. In many cases (e.g. those with a negative diagnosis, metastasis, or lymphoma) subsequent surgery can be avoided. Combining FNA with CNB improved the diagnostic yield in our series compared to either procedure alone without a noticeable increase in bleeding complications.

356 Clinical Significance of Positive Peritoneal Washings Cytology in Patients with Stage I Endometrioid-Type of Endometrial Adenocarcinoma: A Community Hospital Experience

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Background: Peritoneal washings cytology is no longer a part of staging in the most recent International Federation of Gynecology and Obstetrics (FIGO)/TNM classification system 2010 for Endometrial Adenocarcinoma (ECA). However, peritoneal washings are still performed, submitted for cytological evaluation and reported. The literature on clinical significance of positive peritoneal washings cytology (PPWC) shows variable results, making it a controversial issue. Furthermore, there is limited literature evaluating significance of PPWC in Low-Stage ECA (pT1a/b, pN0, pM0). The aim of this study was to evaluate the clinical significance of this finding through cases examined in our institution.

Design: Cases of hysterectomy performed for ECA were retrieved from our files in which peritoneal washings were performed during a 3-year period between 2011-13. Only cases of Endometrioid-type of endometrial adenocarcinoma were included in this study, whereas cases Stage II or higher were excluded. Cytology slides on all cases of PPWC were reviewed. Surgical procedure performed, clinical follow-up and any successive pathologic specimens were reviewed over time period ranging between 22 and 53 months (mean 32 months).

Results: There were 86 patients included in this study set and 8 cases had confirmed PPWC. Patient ages ranged from 37 to 74 years (mean 55 years). The ECA tumor size ranged from 3 cm to 6 cm (mean 4.6 cm). PPWC comprised of three-dimensional groups of atypical glandular cells that were similar to the patient's known uterine primaries. All 8 cases were stage pT1a. There were 3 cases with lymphovascular invasion. The tumor grade was as follows: 3 cases were FIGO I, 3 FIGO II and 2 FIGO III. On chart review of clinical and radiologic follow-up, none of the patients had evidence of recurrent disease or metastasis. All patients had been treated by robotic hysterectomy. None of the patients received any adjuvant therapy.

Conclusions: 1. The incidence of PPWC in ECA in our study is 9.3%, which is consistent with what has been reported in literature. 2. Given that all of the patients in our study were pT1a, PPWC likely represents procedural contamination during robotic surgery, or prior hysteroscopy. 3. Intermediate term follow-up for this study group of Stage I patients of ECA with PPWC suggests that these patients still have a good prognosis. 4. Stage I patients with PPWC are generally not considered for adjuvant therapy and our data lends further support to such a patient management.

357 ALK Rearrangement Fluorescent In-Situ Hybridization Testing for Pulmonary Adenocarcinoma Utilizing Fine Needle Aspiration Smears

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Background: Endobronchial ultrasound guided (EBUS) fine needle aspiration (FNA) biopsy with rapid on-site evaluation (ROSE) is commonly utilized in the evaluation of patients with suspected lung cancer. ROSE is intended to assist in obtaining sufficient material for both primary diagnosis and ancillary testing which includes *anaplastic lymphoma kinase* (*ALK*) rearrangement FISH testing for patients with adenocarcinoma and non-small cell carcinoma, not otherwise specified. *ALK* FISH testing is typically performed on cell blocks however these can be insufficient. The potential alternative use of FNA biopsy smears for *ALK* rearrangement FISH testing was investigated.

Design: 26 EBUS FNA cases were selected and these included 9 benign pulmonary cases and 17 adenocarcinoma cases. The adenocarcinoma cases included 3 *ALK*-positive (ALK-P) and 14 *ALK*-negative (ALK-N) rearrangement cases. Modified Wright stained FNA aspirate slides were evaluated and a technically optimal slide was selected. Areas of visible tumor nuclei and optimal cellular arrangement were marked by a diamond tipped pen. The coverslips were removed and the slides destained. FISH for *ALK* rearrangement at chromosomal locus 2p23 was performed with a FDA-approved Vysis *ALK* Break Apart FISH probe kit (IVD) (Abbott Molecular, Inc. Des Plaines, IL)), using > 15% in 100 cells as a cutoff for a positive result and scoring the cells in the demarcated areas.

Results: Of 3 previous ALK-P adenocarcinomas on cell block, 2 were ALK-P and one was technically uninterpretable on direct smears. 13 of the 14 ALK-N adenocarcinoma correlated and were negative by *ALK* FISH. 1 of 14 previously ALK-N adenocarcinomas was interpreted as ALK-P utilizing the direct smear slide, since it exceeded the cutoff on direct smear. Review of the original cell block result for this case showed several gains in *ALK*, but it did not meet the positive result cutoff. All benign lung cases were technically adequate and ALK-N.

Conclusions: FNA biopsy smears are technically adequate for *ALK* rearrangement FISH testing in pulmonary specimens. Direct aspirate smears for *ALK* rearrangement FISH testing in pulmonary adenocarcinomas are equivalent, and sometimes may be better than *ALK* rearrangement FISH testing on cell blocks. FNA biopsy smears of patient samples can be utilized for *ALK* rearrangement FISH testing.

358 "Indeterminate Cytologic Diagnoses in EUS-Guided FNA of the Pancreas: Confusion or Comprehension?"

Anna Biernacka, Xiaoying Liu, Jonathan Marotti, Edward Gutmann, Thanapoom Boonipat, Timothy B Gardner, Stuart R Gordon, Kerrington D Smith, J Marc Pipas, Vijayalakshmi Padmanabhan. Dartmouth-Hitchcock Medical Center, Geisel School of Medicine at Dartmouth, Lebanon and Hanover, NH.

Background: Indeterminate cytology in EUS-guided FNA of the pancreas represents a diagnostic and management dilemma and it is important that both cytopathologists and treating physicians understand clinical implications involved.

Design: We surveyed cytopathologists and clinicians on decisions triggered by a "suspicious" or "atypical" cytology in EUS-guided FNA of resectable and unresectable pancreatic masses. To assess how indeterminate pancreatic FNAs are managed within our institution, we retrospectively searched the pathology database for "atypical" or "suspicious for malignancy" diagnoses between 2010-15 and reviewed corresponding medical records for follow up.

Results: Thus far, 25 clinicians and 34 pathologists have responded to the on-line survey. There was not uniform agreement either amongst cytopathologists, or clinicians as "how to best handle" indeterminate pancreatic FNA.

EUS-FNA of the Pancreas		Cytopathologists (N=34)		Clinicians (N=25)	
In the setting of a high clinical suspicion of cancer, a cytologic diagnosis:		"atypical cells present"	"suspicious for malignancy"	"atypical cells present"	"suspicious for malignancy"
A. Is sufficient to initiate treatment (surgery vs chemoradiation).	resectable	2 (5.9%)	10 (29.4%)	3 (12%)	6 (24%)
	unresectable	1 (2.9%)	4 (11.8%)	1 (4%)	6 (24%)
B. Always prompts 2nd FNA to establish definitive tissue diagnosis.	resectable	19 (55.9%)	12 (35.3%)	9 (36%)	5 (20%)
	unresectable	21 (61.8%)	14 (41.2%)	10 (40%)	8 (32%)
C. Usually prompts other additional tests.	resectable	8 (23.5%)	4 (11.8%)	6 (24%)	4 (16%)
	unresectable	5 (14.7%)	6 (17.6%)	5 (20%)	1 (4%)
D. Requires decision of multidisciplinary tumor board.	resectable	5 (14.7%)	8 (23.5%)	7 (28%)	10 (40%)
	unresectable	7 (20.6%)	10 (29.4%)	9 (36%)	10 (40%)

In general, cytopathologists perceived that an indeterminate result always prompts a second FNA for definitive diagnosis, whereas clinicians were more likely to defer to the decision of a multidisciplinary tumor board in "suspicious" cases. At our institution, of 540 pancreatic masses evaluated by EUS-FNA, 21 and 41 cases were initially reported as "suspicious" (3.9%) and "atypical" (7.6%), respectively. Only 6 "suspicious" (28.6%; 2 resectable, 4 unresectable) and 16 "atypical" (39%; 2 resectable, 14 unresectable) cases underwent a second EUS-FNA. Furthermore, based on the initial indeterminate cytology, 6 "suspicious" and 8 "atypical" resectable pancreatic masses went directly to surgery, while 3 "suspicious" and 4 "atypical" unresectable patients received chemotherapy and/or radiation (remaining patients had no treatment).

Conclusions: There is no uniform perception of clinical implications of indeterminate diagnoses in EUS-FNA of pancreatic masses. These lesions represent a heterogeneous group and are likely best approached case by case, based on correlation of clinical and pathologic findings. Therefore, good communication between clinicians and cytopathologists is necessary.

359 Indeterminate Thyroid Nodules: Does Asuragen Testing Improve the Specificity of Afirma Test Results?

Shikha Bose, Ann E Walts, Wendy Sacks. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: The Afirma gene expression classifier (Afirma) is an RNA-based assay used to assess risk of malignancy in cytologically indeterminate thyroid nodules [Bethesda categories follicular lesion of undetermined significance (FLUS) and follicular neoplasm (FN)]. Afirma putatively classifies nodules as benign with high sensitivity and negative predictive value (NPV) thus avoiding unnecessary surgery, reducing costs, and improving patient outcomes. However recent studies have reported NPVs from 75-98%. miR*Inform*^{R} Thyroid (Asuragen), a panel of 17 known thyroid cancer mutations and translocations, is also known to improve preoperative diagnostic accuracy for indeterminate thyroid nodules. This study was designed to evaluate the performance of Afirma and to determine if Asuragen testing improved detection of carcinomas.

Design: All thyroid nodules classified as FLUS/FN on FNAs from 2012-2014 with Afirma results and surgical follow-up were reviewed. Of the 4292 thyroid FNAs evaluated during this period, 472 (11%) were diagnosed as FLUS and 86 (2%) as FN. Diagnostic Afirma results were available in 115 cases (93 FLUS, 22 FN). 49 (43%) were categorized as benign and 66 (57%) as suspicious. Surgical follow-up was available for 5 of the Afirma benign and 37 of the Afirma suspicious cases. Asuragen results were also available in 13 of these cases (2/5 with benign and 11/37 with suspicious Affirma results).

Results: Afirma results and surgical follow-up are correlated in Table 1.

	Excision results	
Afirma (no. excised)	Benign	Malignant
Benign (5)	1	4
Suspicious (37)	22	15

Afirma incorrectly classified 4 carcinomas as benign. Asuragen results, available in 2 of these, showed no mutations.

Case	FNA diagnosis	Final Diagnosis	Size of Carcinoma (cm)
1	FLUS	FV PTC	3.8
2	FLUS	FV PTC	4.6
3	FLUS	PTC	0.5
4	FN	Follicular Carcinoma	4.5

One additional cases classified as suspicious by Afirma and negative by Asuragen had papillary carcinoma on excision. Taking into account the 13% rate of malignancy in our indeterminate FNAs, Afirma had a 82% sensitivity, 87% NPV, 52% specificity and 41% PPV in our cohort.

Conclusions: The sensitivity and NPV of Afirma, 82% and 87%, respectively, in our study cohort, vary based on the institutional indeterminate rate and should be determined prior to recommending further management. Additional Asuragen testing did not improve the cancer detection rate of Afirma.

360 Quality Management of Thyroid Fine Needle Aspiration: Inter and Intra-Pathologist Diagnostic Variation

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Background: Fine-needle aspiration (FNA) biopsies with cytological interpretation are the initial step and gold standard towards triage of thyroid lesions. Due to the complexity and nuances in thyroid cytology there has become a need for increased quality management strategies in order to stay in line with The Bethesda System for Reporting Thyroid Cytopathology (TBS). Atypia of undetermined significance (AUS), an equivocal category of TBS has shown wide intra and inter-institutional variation (2-45%). Therefore, we chose to examine our pathologists' AUS rates over time.

Design: Previously collected data on 11,481 thyroid FNA cases between 1/1/12-12/31/14 was reviewed. AUS percentage and AUS:Positive for Malignancy (AUS:M) rates were tabulated by de-identified pathologist and year. Eight cytopathologists consistently signed-out thyroid FNAs over the 3 year period. Two pathologists, lacking data for the full 3 years, were excluded from individual analysis.

Results: There were a total of 976 AUS cases. The overall AUS percentage and AUS:M were 8.5% and 2.0 respectively. The pathologists' AUS percentages ranged from 4.0-11.7% in 2012, 6-10.8% in 2013 and 6.5-12.9% in 2014.

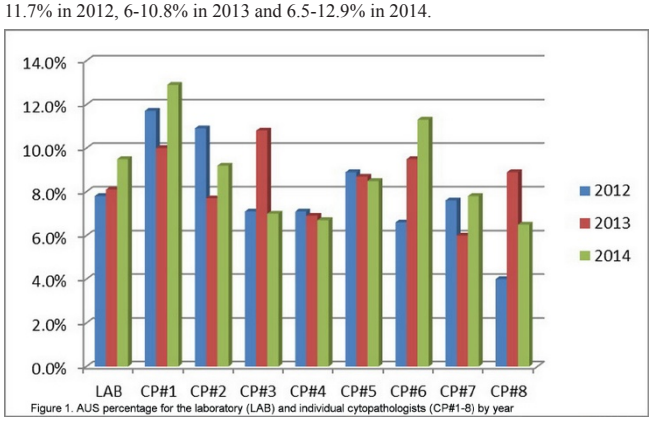

Figure 1. AUS percentage for the laboratory (LAB) and individual cytopathologists (CP#1-8) by year

The AUS:M ranged from 1.0-2.4 in 2012, 1.0-2.8 in 2013 and 1.3-3.3 in 2014.

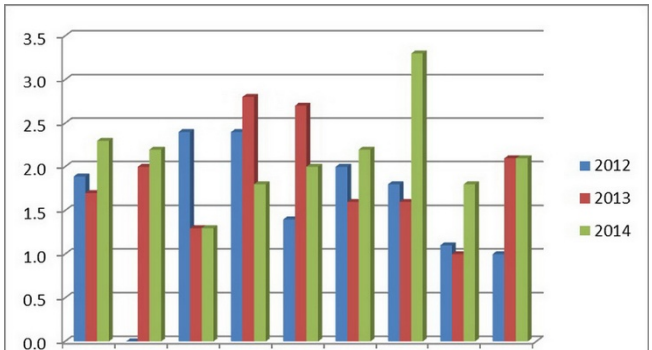

Figure 2. AUS:M for the laboratory (LAB) and individual cytopathologists (CP#1-8) by year

Conclusions: Our study demonstrates that AUS and AUS:M rates vary by pathologist. Despite the slight variation between pathologists and within the same pathologist over time, our institution's overall AUS and AUS:M rates remained relatively consistent over time. Our individual pathologist and overall AUS:M rates remained within or near the recommended 1.0-3.0. Therefore, our study suggests that AUS and AUS:M rates contribute to quality management of thyroid FNAs and provides the rational for future examination of AUS as compared to the other TBS categories in order to determine whether AUS:M or AUS:other TBS category is the best standard for quality measurement of thyroid FNA.

361 Bile Duct Brushings: Accuracy and Interobserver Agreement

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Background: The purpose of bile duct brushing (BDB) cytology is to identify patients with biliary obstruction caused by pre-malignant/malignant diseases, so that surgery may be offered to those with resectable lesions or palliative care for those deemed non-operative. A narrative review has reported an overall sensitivity of 41.6% and negative predictive value of 58%. We investigated whether a multiple tier BDB classification provides more useful information than a 2 category test.

Design: Meditech was searched from 2011-2013 for consecutive BDB cases. Cases were randomly divided among 3 pathologists who classified BDB as non-diagnostic, negative, atypical, suspicious, or malignant. Reference standard was presence of premalignant/malignant disease, as identified on tissue biopsy or resection. Test readers were blinded to the reference standard and vice versa. For analysis of test accuracy, if a patient had multiple cytologies, the first performed BDB result was used. For test agreement among pathologists, 117 cases were read in triplicate (postulated moderate agreement, kappa 0.5, upper limit 0.4, lower limit 0.6).

Results: We identified 222 BDB cytology cases from 191 patients; 3 cases classified as non-diagnostic were excluded. Weighted kappas, for agreement between 2 pathologists, were 0.52, 0.57, and 0.79. Likelihood ratios for a positive test in the following categories were: negative 0.73 (0.48-1.1), atypical 1.47 (0.87-2.48), suspicious 0.93 (0.47-1.48), malignant 1.19 (0.66-2.14). Sensitivity, with positive test defined as atypical, suspicious, or malignant, was 0.68 (0.56-0.79) and specificity 0.44. (0.35-0.53). For every 1000 patients, there would be 376 false positive tests, possibly leading to unnecessary surgical procedures and attendant complications, and 106 false negatives, with possible inappropriate management and decreased survival.

Conclusions: Whether improving agreement among pathologists is possible, and can improve test accuracy, warrants investigation. Otherwise, the limited accuracy of BDB, even with a multiple tier classification, calls into question the usefulness of this procedure.

362 The Performance of the Paris System for Reporting Urine Cytology (PSRUC) in Lower and Upper Tract Specimens: A Comparative Study of 358 Cases

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Background: The interpretation of upper urinary tract cytological specimens is notoriously difficult. We compared the performance of the PSRUC in upper versus lower tract specimens to assess if the proposed criteria perform equally in both settings or not.

Design: 250 lower (LT) and 108 upper tract (UT) cytological specimens with a histological follow-up of 12 months were randomly selected from two institutions. Cytological slides were blindly reviewed and assigned to one of the PSRUC categories: benign, atypical urothelial cells (AUC), suspicious for high-grade urothelial carcinoma (SHGUC), and positive for high-grade urothelial carcinoma (PHGUC). Histological follow-up was recorded as benign, low-grade neoplasia (LGN), or HGUC.

Results: Comparable association rates of the different cytological categories with subsequent HGUC were noted in LT and UT specimens. In the LT, the risk of subsequent HGUC was 11% for the benign, 45% for the AUC, 78% for the SHGUC and 87% for the PHGUC cytology categories. In comparison, the risk was 13% for the benign, 41% for the AUC, 89% for the SHGUC and 86% for the PHGUC cytology categories in UT specimens.

Cytological Diagnosis, Lower tract (n=250)	Benign (n=131)	AUC (n=47)	SHGUC (n=23)	PHGUC (n=45)	Unsatisfactory (n=4)
Histological Diagnosis					
Benign	88	19	3	3	1
LGN	29	7	2	3	2
HGUC	14	21	18	39	1
Predictive Value for HGUC	11%	45%	78%	87%	25%
Cytological Diagnosis, Upper tract (n=108)	Benign (n=46)	AUC (n=22)	SHGUC (n=9)	PHGUC (n=28)	Unsatisfactory (n=3)
Histological Diagnosis					
Benign	28	7	1	1	1
LGN	12	6	0	3	0
HGUC	6	9	8	24	1
Predictive Value for HGUC	13%	41%	89%	86%	33%

Conclusions: Those preliminary results demonstrate that the PSRUC performs comparably in the upper and lower tract specimens. Applying the proposed morphological criteria of the PRSUC results in a significant prognostic stratification of cases into three main cytological groups: benign, AUC, and SHGUC/PHGUC, with an average predictive value for HGUC of 12%, 43%, and 85%, respectively.

363 Sex and Genetic Alterations in High Grade Urothelial Carcinoma on Urine Cytologic Specimens

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Background: Urothelial carcinoma (UC) is the most common form of urinary bladder malignancy. It is estimated that in 2015 there will be 74,000 new cases, including 56,320 occurring in males, making it the fourth most common cancer in males. Although UC is known to have a male sex predilection (~75% of cases), to our knowledge very few authors have studied whether genetic alterations differ between sexes. We aimed to evaluate the genetic alterations in high grade urothelial carcinoma in males and females using urine cytology specimens.

Design: Fifty-three slides from forty-two patients (26 males and 16 females) with cytologic diagnosis and histopathologic confirmation of high grade superficial non-muscle invasive UC were included for this study. All specimens were cellular and contained at least 1,000 cells neoplastic each and the tumor cells represented over 50% of the specimen cellularity. DNA was extracted from ThinPrep cytologic slides and subjected to next generation sequencing (NGS) analysis using a customized targeted exome capture assay composed of 341 genetic abnormalities, including oncogenes, tumor suppressor genes, and components of pathways deemed actionable by targeted therapies.

Results: We discovered genetic alterations involving three genes, MED12, KDM6A and TP53, that either reached or approached a statistically significant difference in prevalence between sexes. These results are outlined below:

	Female		Male		
Mutation	# of cases	Prevalence (%)	# of cases	Prevalence (%)	p-value
MED12	4	25.0	0	0.0	0.043
KDM6A	8	50.0	6	30.0	0.058
TP53	3	18.8	14	70.0	0.055

Conclusions: For men and women, differences exist at a genomic level in UC. TP53, a known tumor suppressor gene was more commonly seen in UC in males, while alterations in KDM6A, a gene located on the X chromosome which most likely also acts as a tumor suppressor gene, is more common in females. Interestingly, mutations in MED12, a gene commonly mutated in uterine leiomyomas and uterine leiomyosarcomas, were only observed in females. Further studies need to be conducted to understand the role these genomic alterations have in tumor biology to determine if separate treatment options need to be explored.

364 Correlation of Fine-Needle Aspiration (FNA) and Frozen Section (FS) Interpretations with the Final Pathological Diagnosis in Thyroid Lesions: Possible Combined Utility of FNA and FS in Managing Thyroid Lesions

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Background: Fine needle aspiration (FNA) is the first line method in evaluating thyroid nodules. If the decision is made to surgically operate, an intraoperative frozen section (FS) is usually requested by the surgeon to further guide management. While the goal is to avoid unnecessary surgery, the utility of FS in the intra-operative surgical management of thyroid lesions with previous FNA evaluation is controversial. The aim of this study is to correlate the findings of FNA, FS and final pathology to assess the combined utility of FNA and FS.

Design: All surgically treated thyroid nodules with a prior FNA evaluation in our institution between 2013 and 2015 were reviewed. FNA, FS, and final diagnoses were collected and categorized. Diagnostic accuracy of FNA and FS and their utilization in surgical management was evaluated and compared.

Results: 130 cases of thyroid surgeries with previous FNA evaluations were identified. Among them, 89 cases (68%) had intraoperative FS evaluations (table 1). There were a total of 74 total thyroidectomies, 47 lobectomies, and 9 initial lobectomies with subsequent completion thyroidectomies.

FNA Diagnosis (n=130)		Non-diagnostic (n=3)	AUS (n=11)	BFN (n=47)	SFN (n=29)	SFM (n=9)	Malignant (n=31)
Frozen Section Diagnosis	No Frozen	0	3	7	5	4	22
	Favor Benign	3	8	37	13	0	0
	FN/De-ferred	0	0	1	10	3	0
	SFM	0	0	0	0	0	0
	Malignant	0	0	2 (1mPTC, 1FTC)	1 (ATC)	2 (PTC)	9 (8PTC, 1MTC)
Final Histology Diagnosis	Nodular Hyper-plasia	3	6	44	14	1	0
	Follicular Adenoma	0	3	0	7	1	0
	Follicular Carcinoma	0	1 (MI-FTC)	1 (WI-FTC)	2 (MI-FTC)	2 (1MI-FTC, 1WI-FTC)	0
	Papillary Carcinoma	0	1	2 (1mPTC, 1FVPTC)	4 (2mPTC, 2PTC)	5	31 (28PTC, 2MTC, 1ATC)
	Other	0	0	0	2 (1ATC, 1Schwan-noma)	0	0

AUS=Atypia of Undetermined Significance. BFN=Benign Follicular Nodule. SFN=Suspicious for Follicular Neoplasm. SFM=Suspicious for Malignancy. FTC= Follicular Thyroid Carcinoma. mPTC=Papillary Thyroid (micro)Carcinoma. MTC=Medullary Thyroid Carcinoma. MI=Minimally Invasive. WI=Widely Invasive. FV=Follicular Variant. ATC=Anaplastic Thyroid Carcinoma.

Conclusions: In the SFM and Malignant categories of thyroid FNA, the malignancy rate was 95% in the final histology diagnoses and FS was unnecessary. FS was used most frequently in the AUS, BFN and SFN categories (72-85% cases) but added very little to the management. Overall, FS altered management in 3 out of a total 89 cases (3%).

365 Alcohol-Fixed Direct Smears Yield High Quality Next-Generation Sequencing Data

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Background: Sample type and adequacy are major factors in the ability to perform next-generation sequencing (NGS). For cytology specimens, most labs only accept FFPE cell block (CB) material for molecular oncology testing. However, this presents multiple problems, including DNA cross-linking and fragmentation, limited cellularity of FFPE CB and variability in CB preparation, which often results in repeat biopsy despite the availability of direct smears (DS). A few studies have reported FNA smears to be an adequate substrate for molecular testing. However, none have reviewed the cellularity requirements for testing nor have they done a direct comparison of smear-based NGS results with FFPE CB-based NGS results.

Design: 18 cases of matched archival FFPE CB and alcohol-fixed DS were collected. One CB H&E and one pap-stained DS for each case was digitally scanned (Aperio) and number of nuclei were counted using Definiens software. DNA was extracted from 1 DS versus 10 unstained slides (10 microns) from CB. DNA was quantified by fluorometry. Libraries were created using 5 cases of DS versus CB using variable input amounts (20ng, 50ng, 100ng, and 250ng) and quantified prior to hybrid capture. Library yield and sequencing results were then compared between DS and CB material. Capture-based NGS was performed targeting the coding regions of >500 cancer genes and select introns covering a total of 2.8 Megabases.

Results: On average, 10-15 times as many nuclei were present in 1 DS (355,463) as compared to 1 CB H&E (26,278). Using equivalent amounts of input DNA for library preparation, DS yielded 2-2.5x as much adaptor-ligated library as compared with CB material (p-value <0.05 for each input amount). Equivalent amounts of library mass were captured and sequenced.

Library yield from FFPE cell block versus direct smear at varying DNA inputs				
	20 ng Input	50 ng Input	100 ng Input	250 ng input
FFPE CB	36 ± 6	76 ± 20	136 ± 86	265 ± 68
Direct Smear	70 ± 21	191 ± 76	310 ± 80	515 ± 141

Conclusions: Cellularity of a single DS was consistently 10-15 fold higher than the cellularity in an FFPE CB HE section. DNA extracted from DS demonstrated statistically increased library yields as compared with DNA extracted from FFPE CB. Sequencing data prepared from direct smears is comparable in quality to that from FFPE cell blocks, and in some cases demonstrated superior results.

366 Molecular Profiling of Malignant Pleural Effusion in Metastatic Non-Small Cell Lung Carcinoma

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Background: This report surveys the molecular diagnostic yield of 50 cases of non-small cell lung cancer (NSCLC)-associated malignant pleural effusion (MPE) and evaluates the potential influence of MPE volume on various cellblock characteristics crucial to the analytic sensitivity of our assays.

Design: In this retrospective analysis, we surveyed the molecular results of cytopathology cases with diagnostically confirmed NSCLC-associated MPE during a 5-year period from January 2010 to January 2015. Cases where molecular testing was requested but not performed were also included, provided an explanation was documented or readily inferred. All cases had their cellblock characteristics reviewed without knowing the molecular outcome. Our molecular profiling suite consisted of fluorescent in situ hybridization (FISH) for *ALK* gene rearrangements, Sanger sequencing (for *EGFR*) and high-throughput pyrosequencing (for *KRAS* and *BRAF*) from January 2010 to December 2013, and targeted next-generation sequencing (NGS) performed thereafter (ie, starting In January 2013) in the study period. All molecular tests were performed on DNA from formalin-fixed, paraffin-embedded (FFPE) MPE-derived cellblocks.

Results: 50 cases of NSCLC-associated MPE were identified where molecular testing was requested either by the diagnosing cytopathologist or by the patient's clinician. Of these, 14 cases (28%) were rejected due to inadequate tumor cellularity. 27 MPE cases (54% of the total) underwent full molecular testing (define as at least *EGFR* and *KRAS* sequencing and FISH for *ALK* rearrangement). 6 cases had *ALK* FISH only. 3 cases were not available for blinded review of their cellularity. Of the 27 cases with full molecular testing results, a genetic abnormality was detected in 16 cases (59%). The most common genetic aberrations identified involved *EGFR* and *KRAS* (9 and 7 cases, respectively). An *ALK* rearrangement was found in 1 of the cases that had only ALK FISH performed. MPE volume was not associated with overall cellularity or tumor cellularity. The cases that were rejected for both sequencing and FISH due to inadequate tumor cellularity were not statistically different in MPE volume from cases that underwent full molecular testing (P=0.360).

Conclusions: Molecular profiling of MPE is a viable alternative to testing solid tissue in advanced NSCLC. After streamlining our selection of cases to minimize the risk of false negatives from inadequate tumor cellularity, our methods were able to successfully detect genetic aberrations in 59% of samples that underwent full molecular testing.

367 Trends in Rates of Failure of Care in Women with Invasive Cervical Cancer: A Meta-Analysis

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Background: Cervical cancer occurs in spite of successful preventive measures including screening, vaccination and treatment of precursors. The reasons of failure of care in this situation are generally classified into (1) inadequate screening, (2) never screened (3) missed opportunity (4) failure of detection or (5) failure of follow-up. In the last two decades or so, new methods of screening and management of cervical cancer precursors have been introduced. The impact of these changes has not been assessed on reported rates of reasons for care failure. In this meta-analysis we assess trends of rates of failure of care in women with invasive cervical cancer in the past five decades.

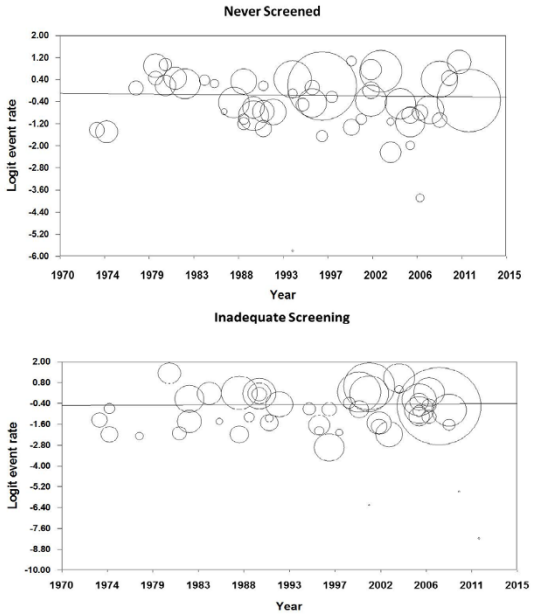
Design: This meta-analysis included all studies reported in PubMed in the period 1975 to 2016. Search terms included "invasive cervical cancer", "screening" and "follow-up". Manuscripts were reviewed and included for further analysis if failure of care was attributed to one of the above reasons. Random effect rates and associated confidence interval (CI) were calculated using Comprehensive Meta-Analysis software. Trend of rates over the years was assessed using meta-regression. Results were considered significant at p =0.05.

Results: 75 out of 1727 studies were identified using the selection criteria. Inadequate or no screening remained the main reasons for failure of care.

Reason for care failure	Rates	Number of studies
Never screened	39% (C.I. 37-44%)	51
Inadequate screening	31% (C.I. 25-37%)	56
Missed opportunity	23% (C.I. 17-29%)	9
False negative/ Failure of detection	18% (C.I. 15-21%)	44
Failure of follow-up of abnormal tests	39% (C.I. 34-44%)	53

These rates have not significantly changed over the last 50 years.

Figure: Trend of rates of never screened and inadequate screening for cervical cancer. The size of the bubble represents the study size



Conclusions: In spite of substantial improvement in the way we screen for cervical cancer in the last two decades, the patterns of care failure remained the same over the last five decades with the majority of care failure attributed to inadequate or lack of screening.

368 Candida and Trichomonas and High-Risk HPV Infection

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Background: The diagnosis of cervical epithelial abnormalities on a Pap smear (PS) is an extremely important procedure which can be impaired by intrinsic specimen limitations. Infections with *Candida* and *Trichomonas* species can induce cellular atypia, through inflammation and inflammation-related changes, complicating diagnosis of dysplasia.

Design: The study examined the results of PS from 2013-2015 (n= 19,927) to determine if there is a link between *Candida*, *Trichomonas* and HPV infection. As a secondary endpoint, the study analyzed the relative frequency of positivity of high-risk HPV in patients with and without infections and also monitored the subsequent PSof these patients.

Results: There were 18276 (92%) PS negative for *Candida* and *Trichomonas*. Of these, 5055 (28%) were tested for HPV and 665 (13%) were positive. Of the 1231 PS with *Candida*, 302 (25%) were tested for HPV and 55 (18%) were positive. Of the 420 PS with *Trichomonas*, 123 (29%) were tested for HPV, of which 13 (10%) were positive. Of the 665 HPV+ PS without *Candida* or *Trichomonas*, 209 (31%) were diagnosed as negative, 336 (51%) as ASCUS, 14 (2%) as ASC-H, 107 (16%) as LSIL and 30 (5%) as HSIL. Of the 247 HPV- PS with *Candida*, 184 (74%) were diagnosed as negative, 60 (24%) as ASCUS, 1 (<1%) as ASC-H and 2 (<1%) as LSIL. Of the 55 HPV+ PS with *Candida*, 19 (34%) were diagnosed as negative, 21 (38%) as ASCUS, 2 (3%) as ASC-H, 11 (20%) as LSIL and 2 (3%) as HSIL. Of the 110 HPV- PS with *Trichomonas*, 91 (83%) were diagnosed as negative, 18 (16%) as ASCUS and 1 (<1%) as ASC-H. Of the 13 HPV+ PS with *Trichomonas*, 10 (77%) were diagnosed as negative, 2 (15%) as ASCUS and 1 (8%) as LSIL. Of the HPV+ PS with ASCUS and *Candida*, 20 had followup. Seven of these (35%) had progressed to LSIL/HSIL. Of the HPV positive PS with *Candida* with a negative diagnosis, 7 had followup. Of these 2 (28%) had progressed to LSIL/HSIL.

Conclusions: Women with Candida infection were more likely to have a concurrent positive test for high-risk HPV (18%) than patients without *Candida* infection (13%). In addition, HPV+ patients with *Candida* and T*richomonas* are more likely to be diagnosed with "inflammatory changes" (34% and 77% respectively) vs HPV+ patients without *Candida* or *Trichomonas* (31%). Followup also revealed that seven HPV+ ASCUS and 2 HPV+ negative PS patients with *Candida* infections progressed to LSIL and HSIL. There may be an increased incidence of high-risk HPV infection in women with concurrent infection with *Candida* or atypia might be attributed to *Candida*-induced inflammation rather than dysplasia.

369 Atypical Squamous Cells, Cannot Exclude High Grade (ASC-H), High Risk Human Papillomavirus (hrHPV)-Negative Risk Group: Should There Be an Alternative Management?

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Background: The ASCCP consensus guidelines recommend immediate colposcopy, regardless of the age or hrHPV status, for woman diagnosed with ASC-H. Herein we analyze a large retrospective cohort of ASC-H positive patients and correlate their follow-up histologic diagnosis to HPV status so as to identify trends for management implications.

Design: A computer-based search was used to identify ASC-H with concomitant hrHPV testing from 1995-2015. hrHPV DNA testing was ordered by clinicians either as reflex or cotest. Histopathologic follow-up included endocervical curettage, cervical biopsy/conization using a loop electrosurgical excision or cold-knife was recorded. Patient's demographics were additionally recorded. Data analysis utilized standard statistical methods.

Results: 1334 cytology cases diagnosed as ASC-H were identified. The average age was 35.5 years (15-87). 98.8% (1328) were tested for the presence of HR-HPV either as reflex (64.3%) or as a cotest (35.6%) with 67% positive. The biopsy rate was similar between HPV positive ASC-H (60.3%) and HPV negative (55.6%). The outcome of a benign biopsy in the HPV negative group was 73.1% versus only 33.5% in the HPV positive group ($p<0.0001$). The outcome of HSIL on biopsy among HPV positive was 33.1% versus 9.5% for HPV negative ($p<0.001$). The rate of LSIL among HPV positive was 21% versus 7% among HPV negative ($p<0.001$). There was no statistical difference in these biopsy outcomes when stratified by age (< or > 30) or type of HPV test. Although the reflex negative HPV (47.8%) had a lower rate of biopsy follow-up relative to cotest negative (66.5%) there was not a statistically significant difference in the outcome of a SIL on biopsy 17.8% (reflex) versus 15.5% (cotest) ($p<0.3$).

Conclusions: The ASC-H category is an uncommon and equivocal interpretation with the goal of identifying patients with a higher risk of HSIL than those with ASC-US. The low-reproducibility of the ASC (-US and –H) diagnostic categories places significant emphasis on the management of these equivocal groups. The utility of hrHPV testing has made a significant impact in cervical cancer diagnosis but in particular, has aided clinicians in better triaging patients in these controversial risk groups into more appropriate management algorithms. Our findings would support the clear importance of hrHPV triage, specifically with respect to an ASC-H diagnosis.

370 The Diagnostic Accuracy of Thyroid Fine Needle Aspirates for Atypia, Suspicion for Malignancy and Malignancy in Hashimoto Thyroiditis, a Single Institutional Experience

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Background: Hashimoto thyroiditis is a diagnostically challenging entity on cytology. The follicular atypia present in the context of Hashimoto thyroiditis can often result in a high false positivity rate of atypia on fine needle aspirates (FNA).

Design: A computer-based search was carried out to identify all cases diagnosed in accordance with the TBSRTC categories as atypia of undetermined significance (AUS), suspicious for follicular neoplasm, suspicious for malignancy and malignant in Hashimoto thyroiditis from 2005-2015. Histopathologic follow-up from thyroid lobectomy or thyroidectomy was recorded and correlated with cytology. Patient demographics, number of FNAs, and nodule size was recorded. Standard statistical methods were utilized.

Results: Between 2005-2015 a total of 204 cytology patients (mean age of 45 yo) diagnosed with AUS, suspicious or malignant were identified. 130 cases were categorized as AUS, 43 as suspicious and 31 as malignant. Of these cases, a surgical follow-up of either a thyroid lobectomy or total thyroidectomy was available for 47% (95). Microcarcinomas were excluded. Among AUS cases, 35% had surgical follow-up; 40% were diagnosed with papillary thyroid carcinoma, 49% benign, 7% Hürthle cell neoplasm, 2% follicular carcinoma and 2% lymphoma. Among the suspicious cases 67% had surgical follow-up; 48% were diagnosed with papillary thyroid carcinoma, 34% benign, 14% Hürthle cell neoplasm and 3% follicular neoplasm. Among the malignant cases, 67% had surgical follow up; 86% were diagnosed with papillary thyroid carcinoma and 14% were diagnosed as benign. The positive predictive value (PPV) for AUS is 50% [95% CI 0.36-0.64], suspicious for malignancy is 65% [95% CI = 0.54-0.85] and malignant is 86% [CI 0. 0.65-0.95]. There was no statistically significant difference in the mean size of the nodule between diagnostic categories, AUS-1.4cm; suspicious-1.2cm; malignant-1.0cm ($p<0.7$).

Conclusions: The overall risk of malignancy for the TBSRTC categories within our cohort of Hashimoto thyroiditis are consistent with the implied risks of TBSRTC. Although our cohort AUS malignancy rate appears to be higher relative to TBSRTC and published studies, this may reflect the multidisciplinary approach and slightly lower rate of surgical intervention at our institution. This data would support that a conservative diagnostic approach and the utilization of TBSRTC diagnostic criteria can maintained a high level of accuracy despite the challenges of Hashimoto thyroiditis atypia.

371 False Negative HPV Tests in Women with High Grade Squamous Intraepithelial Lesion or Carcinoma Pap Tests: Clinical-Pathological Correlation

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Background: Human papillomavirus (HPV) testing has been recommended for primary cervical cancer screening in the United States. Knowledge of false negative HPV results in Pap cytology specimens may improve screening efficacy. In this study, we evaluated potential contributing factors to false negative HPV tests in women with a high-grade intraepithelial neoplasia (HSIL) or squamous cell carcinoma (SCC) Pap test result.

Design: We retrospectively searched the database in our institution for women with HSIL or SCC Pap and HPV co-testing results from 2006 to 2014. A total of 175 women were identified with a median age of 47 years (range, 18-88). Pap cytology and HPV co-testing were performed by SurePath Pap and either Hybrid Capture 2 (59%) or Cervista HPV (41%). A total of 139 patients (126 HSIL and 13 SCC) had tissue-confirmed high-grade cervical/vaginal intraepithelial neoplasia (CIN2/VAIN2+). Clinical-pathological information was collected. Logistic regression models were used to assess the differences associated with false-negative HPV tests.

Results: HPV tests were negative in 15% (21/139) of cases. All false-negative HPV results were from patients screened at the Gynecology Clinics (21/121, cancer surveillance) rather than the Cancer Prevention Center (0/18, cancer screening) (P value = 0.0423). Significantly more false-negative HPV results were observed in vaginal Pap specimens and current smokers. Past use of an oral contraceptive was associated with an 84% reduction in odds of a false-negative HPV result (Table 1).

Covariate	Reference	Odds Ratio	p-value
Pap location:Vagina	Cervix	3.675	**0.0099**
Transition Zone:Absent	Present	1.51	0.83
HPV Assay:Hybrid Capture 2	Cervista	0.703	0.46
Dysplasia History(≥CIN2/VAIN2)	None	2.689	0.27
Prior treatment for cervical dysplasia:Yes	None	2.14	0.12
Immunocompromised:Yes	No	1.895	0.45
Oral contraceptive use:Past	No	0.167	**0.0360**
Estrogen replacement use:Past	No	3.111	0.24
Postmenopausal:Yes	No	2.234	0.09
Current smoker:Yes	No	2.935	**0.0310**

Three of 4 patients with bone marrow transplant had a negative HPV result; although this finding is not statistically significant, it suggests that immunosuppresion due to bone marrow transplant may affect HPV positivity.

Conclusions: False-negative HPV tests occur mainly in high-risk patient populations at cancer surveillance clinics. For these patients, the HPV result may be affected by vaginal Pap sampling, smoking, or immunosuppression.

372 Nuclear Size Does Not Predict Clinical Outcomes When Evaluating Thyroid AFLUS by FNA

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Background: Follicular variant of papillary carcinoma (FVPC) is the second most common type of thyroid cancer. Distinguishing benign from malignant follicular neoplasms by fine needle aspiration (FNA) biopsy is very challenging. For example, FVPC is often classified as an atypical follicular lesion of undetermined significance (AFLUS), or suspicious for follicular neoplasm (FN). Since increased nuclear size is one important feature that distinguishes FVPC from other follicular neoplasms in surgical specimens, we hypothesized that the average nuclear size would be larger in the FNA SurePath slides from FVPCs compared with benign thyroid nodules (BTN) and follicular adenomas (FA).

Design: Retrospective review of all 30 thyroid ultrasound-guided FNAs diagnosed as AFLUS, or suspicious for FN, from 2007-2014 with surgical lobectomy outcomes (27/30), or at least 2 years of clinical follow-up. Patient age and gender were recorded. The nuclear diameter in microns (um) of 100 follicular epithelial cells in the SurePath slide of each case was measured using a micrometer while blinded to FNA diagnoses and clinical outcomes. The mean nuclear diameter for each case was reported for analysis. Comparisons between groups were made by unpaired t-test.

Results: Most of the FNAs in this study were from females (26/30) with 17 benign and 13 malignant outcomes (Table). AFLUS predicted thyroid carcinoma in 27% of cases. Suspicious for FN predicted carcinoma in 53%. As expected, Hurthle cell neoplasms (adenoma: HA, or carcinoma: HC) tended to have larger nuclei compared with other follicular neoplasms [p=0.08]. BTN, FAs, the one case of medullary carcinoma (MC), and the eleven cases of FVPC were not significantly different than the group averages.

Cytology/ Outcomes	**Mean Nuclear Diameter (um) +/- Standard Error by Surgical Outcomes**						
	BTN (n=8)	FA (n=7)	HA (n=2)	HC (n=1)	MC (n=1)	FVPC (n=11)	Totals (n=30)
AFLUS (n=11)	6.9 (0.2)	7.2	7.5 (1.0)	8.1	-	5.7 (0.1)	6.9 (0.3)
Suspicious (n=19)	6.0 (0.1)	6.6 (0.3)	-	-	6.4	6.6 (0.2)	6.5 (0.1)
Totals (n=30)	6.6 (0.2)	6.7 (0.3)	7.5 (1.0)	8.1	6.4	6.4 (0.2)	6.6 (0.1)

Conclusions: We conclude that nuclear size does not appear to distinguish FVPC from benign follicular lesions diagnosed as AFLUS or suspicious for FN by thyroid FNA. A different approach, such as molecular testing, may be needed to distinguish malignant diagnoses from benign mimics.

373 CellDetect®, a Novel Urine-Based Bladder Cancer Test: A Multi-Institutional Validation Study

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Background: CellDetect® is a unique histochemical-stain enabling color and morphological discrimination between malignant and benign cells. In an open-label study, we showed the capability of CellDetect® to accurately identify urothelial cell carcinoma in urine smears. The objective of the current study was to validate the findings on blinded settings.

Design: The study was conducted in nine hospitals enrolling patients monitored for history of urothelial cell carcinoma. 217 urine smears, including 96 positive and 121 negative cases, were recruited for the study. Smears were blindly observed by at least two cytology experts, and diagnoses were compared to patients' true diagnoses.

Results: The overall sensitivity of CellDetect® to detect urothelial cell carcinoma was 84%. Notably, this high sensitivity was observed regardless of cancer stage, as 85% of early stages, non-muscle-invasive cancers were detected. Moreover, the accuracy of detecting low-grade tumors was also relatively high (78%). Specificity, tested on subjects undergoing routine surveillance by cystoscopy, was 84%. Follow up that was conducted on CellDetect® false-positive cases showed a significance elevation in the recurrence rate among this group (21%) in comparison to true-negative cases (2%), suggesting that CellDetect® is capable of early detection of urothelial cell carcinoma recurrence.

Conclusions: This study validates the capability of CellDetect® in identifying urothelial cell carcinoma recurrence. Of special importance is that the high sensitivity was maintained throughout all cancer grades and stages. The data suggests that CellDetect® can be further developed to provide a non-invasive alternative to cystoscopic surveillance.

374 Differential Outcomes of Patients with FNA Diagnoses of AUS Versus FLUS: The Potential Need for Separation in the Bethesda System

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Background: The Bethesda System (TBS) for thyroid cytopathology reporting has helped standardize thyroid fine needle aspiration (FNA) reporting and management. In the current version, atypia of undetermined significance (AUS) and follicular lesion of undetermined significance (FLUS) constitute the Bethesda Category III, with combined estimated risk of malignancy of 5-15%, and have similar follow-up. However, the main differential diagnosis for AUS is benign vs. papillary thyroid carcinoma (PTC), a relatively common entity. To the contrary, the differential diagnosis for FLUS includes benign entities such as follicular adenoma, Hurthle cell adenoma, as well as much less common follicular carcinoma (FTC). The aim of this study was to examine the differential outcomes of AUS and FLUS, assess their respective risks of malignancy, and consider whether they should be included in the same TBS category.

Design: An IRB approved retrospective chart review was performed for patients with thyroid FNAs (2010-2014), including cases performed at the University of Washington (UW), and cases reviewed in consultation for subsequent care at UW. From a total of 1233 patients, 119 had a diagnosis of FLUS and 64 had a diagnosis of AUS. Patient records were reviewed for subsequent clinical follow-up. Cases lost to follow-up (17 FLUS and 7 AUS) were excluded. Outcomes for patients with AUS and FLUS were evaluated and results were compared.

Results: 16/57 patients with AUS (28.1%) had carcinomas (93.8% PTC and 6.2% FTC) on thyroidectomy, statistically higher than the 8/102 patients (7.8%, p=0.001) with FLUS and subsequent carcinoma (75% PTC and 25% FTC). Among patients who underwent surgery, those with AUS had statistically higher rates of carcinoma (16/31, 51.6%), compared to those with FLUS (8/32, 33.3%; p=0.0394). 24/159 (15.1%) of patients with either AUS or FLUS had carcinoma on subsequent histology.

Conclusions: The 15.1% risk of malignancy after either AUS or FLUS is in line with TBS estimated risk rates. However, patients with AUS showed ~3.5-fold higher relative risk of malignancy when compared to patients with FLUS. Among patients who had surgery following their FNA, relative risk of malignancy was ~1.5-fold higher for AUS than FLUS. Our data suggest that the diagnoses of AUS and FLUS are associated with different predicted risks of malignancy and that TBS Category III may best be split into two groups, AUS and FLUS, with different implied risks of malignancy. Such a distinction may aid the development of different management strategies for patients with AUS and patients with FLUS.

375 Comparison of Malignancy Rates in Thyroid Fine Needle Aspiration Using the Bethesda Category System in a Tertiary Care Center and a Comprenhensive Cancer Center: Institutional Statistics

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Background: The Bethesda System for Reporting Thyroid Cytopathology is the standard for interpreting Fine Needle Aspiration (FNA) specimens. This system recommends six diagnostic categories. Each category implies a risk of malignancy and a recommended clinical management. Our objective was to determine the rate of malignancy per category in thyroid nodule FNAs in a comprehensive cancer center and a tertiary care academic hospital.

Design: A retrospective review of thyroid cytology FNA specimens with concomitant thyroid resection from 2013 to 2015 at a tertiary care academic hospital (TCAH) and a comprehensive cancer center (CCC) was performed. Occult/incidental microcarcinomas were excluded from this analysis.

Results: Of the 1343 patients with FNA from thyroid nodules, 156 patients had surgical resection (125 females, 31 males) with concurrent 202 FNAs. The mean age was 50 years (range 14 to 80 y/o). Atypia/follicular lesion of undetermined significance (AUS/FLUS; Bethesda category III) represented 38% (77/202), suspicious for follicular neoplasm (Bethesda IV) 7% (15/202), suspicious for malignancy (Bethesda V) 10% (21/202), and malignant (Bethesda VI) 25% (50/202). Malignancy rates for the TCAH and CCC for Bethesda III was 20% and 40%, Bethesda IV was 36% and 0%, for Bethesda V was 100% and 95%; and for Bethesda VI was 100% in both institutions.

Bethesda Category	FNA	FNA	Malignacy Rate	Malignancy Rate	Malignant Rate
	TCAH	CCC	TCAH	CCC	Overall
III (AUS/FLUS)	30 (30%)	47 (46%)	20%	40%	32%
IV (Suspicious for follicular neoplasm)	14 (14%)	1 (1%)	36%	0%	33%
V (Suspicious for malignancy)	2 (2%)	19 (18%)	100%	95%	95%
VI (Malignant)	14 (14%)	36 (35%)	100%	100%	100%
Total	99 (100%)	103 (100%)			

Conclusions: The malignancy rates for Bethesda III (AUS/FLUS) thyroid nodules in our institutions are higher than what has been generally proposed for this category. This higher rate is more evident in the patients seen at our referral comprehensive cancer center. Individual institutional malignancy rates need to be estimated at every laboratory/hospital setting

376 Impact of ThyroSeqv.2 Next-Generation Sequencing Assay on Fine Needle Aspiration Cytology of Thyroid Nodules

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Background: Fine-needle aspiration(FNA) cytology is a common approach to evaluate thyroid nodules,distinguishing benign from malignant nodules in the majority of cases. We compare and note the differences in reporting of thyroid nodules before and after implementing ThyroSeqv.2 at our Institution.This study also assesses whether next-generation sequencing(NGS) assay can offer significant improvement in diagnosis of thyroid nodules.

Design: We compare Bethesda II-VI thyroid nodules before and after implementation of NGS ThyroSeqv.2.The molecular panel required a dedicated additional pass by the Endocrinologist. NGS assay simultaneously detects >400 point mutations and gene fusions >60 thyroid cancer genes to increase the detection of thyroid malignancies. 21% of these nodules had definitive surgical follow up.

Results: Our study included a total of 225 consecutive cases of thyroid nodules in the pre ThyroSeq era.

preThyroSeq cases July2014-Jan2015	Bethesda II	Bethesda III	Bethesda IV	Bethesda V	Bethesda VI
225	170(75.55%)	26(11.55%)	13(5.77%)	12(5.33%)	4(2.08%)

In the post ThyroSeq period a total of 206 nodules were included.

postThyroSeq cases Feb-August2015	Bethesda II	Bethesda III	Bethesda IV	Bethesda V	Bethesda VI
206	125(60.6%)	49(23.7%)	16(7.7%)	8(3.8%)	8(3.8%)

63 cases were subjected to ThyroSeq analysis.In the ThyroSeq period,there was a statistically significant increase in the number of cases categorized as Bethesda III($p<0.001$) and a decrease in Bethesda II($p<0.001$). In the Bethesda II category, 2 of 4 cases showed mutations,one of which on histological analysis revealed a follicular variant of papillary carcinoma.The most frequent mutation associated with malignant nodules were NRAS and BRAFV600E. Among the 13 nodules with surgical follow up, histologic analysis revealed cancer in 10 cases. All cases of Bethesda IV,V,VI when associated with a mutation revealed a thyroid neoplasm on resection.

Conclusions: Implementing ThyroSeq prompted an increased use of the Bethesda III category,without a significant change in the Bethesda IV, V, VI categories.

Bethesda II thyroid nodules with a high clinical suspicion of malignancy should be subjected to molecular analysis.

A cytologic diagnosis of Bethesda IV,V,VI in conjunction with a mutation is highly predictive of a thyroid neoplasm.

NGS assay was accurately able to predict cancer in 77% cases,with a mutation on FNA sample, as seen with surgical follow up, thereby making ThyroSeqv.2 a powerful tool to guide clinical management and avoid repeat FNA.

377 HER2 FISH Concordance in Breast Cancer Patients with Both Cytology and Surgical Pathology Specimens

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Background: Fluorescence in Situ Hybridization (FISH) for Human Epidermal Growth Factor Receptor 2 (HER2) is traditionally performed on histologic specimens. Although HER2 FISH is performed on cytologic specimens in the clinical setting, there are no specific guidelines for validation of HER2 testing in these samples. Our aim was to correlate HER2 FISH in ThinPrep and cell block cytology specimens with HER2 FISH in histologic samples.

Design: We identified 54 breast carcinomas in which HER2 FISH had been performed on both cytology and surgical pathology specimens. An FDA-approved dual probe HER2 FISH assay was performed using probes for the HER2 (ERBB2) locus and the alphacentromeric region of chromosome 17 (CEP17) and interpreted using updated 2013 ASCO/CAP guidelines.

Results: Cytology specimens were from 54 women (34 to 98 years old) and included 47 metastatic and 7 primary breast carcinomas. Carcinomas were diagnosed by FNA

in 41 cases and on fluid cytology in 13. HER2 FISH was performed on 32 cell blocks and 22 ThinPrep slides. In the surgical specimens, HER2 FISH was performed on the primary breast carcinoma in 47 (87%) cases, and on a metastasis in 7 (13%) cases. Of the 54 cytology specimens, the HER2 FISH results were negative in 43 (80%), positive in 8 (15%), and equivocal in 3 (5%). Of the 43 cases with a negative result on a cytology specimen, the surgical specimen was also negative in 40 (93%) and positive in 3 (7%). Of 8 cases that were HER2 FISH positive on a cytology specimen, 5 (63%) were positive on a surgical specimen and 3 (37%) were negative. All 3 equivocal cases on cytology were negative on a surgical specimen. Overall, 48 of 54 cases (89%) had a concordant result, while 6/54 (11%) were discordant. In patients with a discordant result, the average interval between HER2 FISH testing of the primary tumor (surgical specimen) and the metastasis (cytologic specimen) was 7 years (range 2-13). Three of 6 (50%) discordant cases showed HER2 genetic heterogeneity in the cytology specimen. Five patients with a discordant result received adjuvant chemotherapy, 3 received endocrine therapy, and 1 received HER2-targeted therapy with trastuzumab.

Conclusions: HER2 FISH is a reliable method for determining HER2 status in ThinPrep and cell block cytopathology specimens. Discordant results between cytologic and surgical specimens are uncommon; possible explanations include genetic heterogeneity or other changes that occur in the interval between primary tumor diagnosis and testing of the metastasis.

378 Trichomonas vaginalis Is an Under-Described Infection in Urine Cytologies from Men

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Background: *Trichomonas vaginalis* (TV) causes a common sexually transmitted infection in women, and organisms found in urine cytology specimens are assumed to be vaginal contaminants. TV infection in male urine cytologies, however, is not well recognized, and only rare single cases have been reported in the literature. Untreated TV infection in men may lead to adverse outcomes, including urethritis, epididymitis, prostatitis and infertility, in addition to sexual transmission to their partners. The aim of this study was to determine the prevalence and significance of *TV* detected by urine cytology in men.

Design: Our cytopathology archives were queried for urine cytology specimens containing *Trichomonas* over a period of 20 years (1985–2015). Clinical information in men with *Trichomonas*-positive urines was reviewed retrospectively. Where available, the presence and morphology of organisms, and the associated inflammatory reaction was recorded.

Results: Of approximately 60,000 urine cytology specimens, we identified 73 (0.1%) that contained *TV*, including 2 PCR-confirmed cases. Of these, 28 (38%) were from male patients ranging in age from 28 to 87 years (mean 67, median 71). Clinical history and treatment information was available in 12 of 28. Lower urinary tract symptoms were reported in 10, the most common being hematuria (6). Less common symptoms included nocturia, frequency, urgency, hesitancy, and incontinence. Five patients had an elevated serum prostate specific antigen (PSA). Urethral strictures were found by cystoscopy in 2 patients. Two patients were found to have *TV* in urine after their wives were diagnosed with *TV*. Eight (8/12, 75%) were treated with metronidazole. Slides were available for review in 7/28 cases. *TV* ranged from few (3 per high power field) to numerous (30 per high power field). They showed typical morphologic features including round to oval shape, a single eccentrically placed nucleus and cytoplasmic granules. An associated acute inflammatory reaction was seen in 4 cases.

Conclusions: To our knowledge, this study is the largest series of *TV* infection in male urine cytologies in the literature. Although TV infection is exceedingly rare in urine samples, male specimens made up a surprisingly high proportion. Most men with *TV* infection had no prior diagnosis, and received specific antibiotic therapy based on urine cytology results, suggesting that the infection was clinically significant. Urine cytology may represent the first diagnostic test of TV in males, and could prevent undesired adverse outcomes if infection went undetected and untreated.

379 GATA-3 in the Work-Up of Malignant Effusions: A Cell Block Micro-Array Study

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Background: GATA-3 is a transcription factor involved in the differentiation of human tissues. It has proven to be a successful marker for breast and urothelial carcinoma. We aim to evaluate the role of GATA3 in the work up of malignant effusions and the diagnosis of breast carcinoma in fluids.

Design: Following IRB approval the laboratory archives were searched for cell blocks from malignant effusions with confirmed diagnosis. A total of 100 cases were selected; 28 breast carcinoma, 26 lung adenocarcinoma, 15 gastrointestinal adenocarcinoma, 23 Müllerian adenocarcinoma and 8 reactive mesothelium. Cell block micro-arrays (TMA) were constructed with 3 cores from each cell block using a 1 mm core needle. Microarray slides were stained for GATA-3, ER and PR. Sensitivity and specificity of GATA3 was calculated and Fisher exact test was used to analyze the relationship between variables.

Results: All cases were well represented in the TMA sections. Positive nuclear staining was identified in 24/28 (86%) of breast carcinoma cases (p value=0.0001). All non breast cancer cases were negative for GATA-3 with the exception of one Müllerian adenocarcinoma. GATA3 has a specificity of 99%. ER was positive in 20/28 (71%) of breast carcinoma cases. PR was positive in 16/28 (57%) of breast carcinoma cases. Of the 24 GATA-3 positive cases, 19/24 (79%) were ER positive, 15/24 (63%) were PR positive, 14/24 (58%) were ER/PR positive, 5/24 (21%) were ER positive PR negative and 1 (4%) was PR positive ER negative. One case showed weak ER/PR positivity while GATA-3 was negative. ER is positive in 16/23 (70%) of Müllerian adenocarcinoma cases, and 1/26 (4%) of lung adenocarcinoma cases. PR is positive in 1/8 reactive mesothelial cases, 14/23 (61%) of Müllerian adenocarcinoma cases, and 2/15 (13%) of Gastrointestinal adenocarcinoma cases. In addition, GATA-3 expression was not seen in background mesothelium in any of the cases but was appreciated in lymphocytes.

Conclusions: GATA3 is a useful marker in the workup of malignant effusions when metastatic breast carcinoma is in the consideration. GATA 3 was positive in all ER positive cases with the exception of one case. In addition, GATA-3 was also reactive in 5 ER negative cases.

380 Interobserver Variability of Cytotechnologists in Papanicolaou Smears of Glandular Lesions of Cervix

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Background: The interobserver variability of papanicolaou smear diagnosis of atypical glandular cells of undetermined significance (AGUS) between cytopathologists has been reported to be very poor. Given that our cytotechnologists play an important role in screening our papanicolaou smears, we sought to determine the interobserver variability of our cytotechnologists in diagnosing glandular lesions.

Design: Fifty cases with an initial diagnosis of atypical glandular lesions (AGUS) on thin prep smears with documented histologic follow-up were included in the study. Twenty-nine cases negative for intraepithelial lesion or malignancy (NILM) with corresponding histologic follow-up were also included to serve as a control. Blinded to the diagnosis and histologic outcome, 5 cytotechnologists were asked to diagnose the slides based on the Bethesda System for Reporting Cervical Cytology. The interobserver agreement with the histological gold standard was calculated using the kappa statistics by Landis and Koch.

Results:

Bethesda Classification	Kappa (SD)
Atypical glandular lesion of undetermined significance (AGUS) or higher	.43 (0.15)
Squamous intraepithelial lesions (SIL) or higher	.51 (0.25)
NILM	.61 (0.16)

For our results, the interpretation of kappa value was as follows: <0 no agreement, 0-0.20 slight, 0.21-40 fair, 0.41-0.60 moderate, 0.61-0.80 substantial and 0.81 to 1.0 almost perfect. For these cases and each category, the kappa agreement was moderate to substantial. McNemar's chi-square test statistic showed that the cytotechnologists neither under nor over diagnosed negative smears. However, they underdiagnosed SIL ($p<0.05$) and overdiagnosed AGUS ($p<0.05$). All kappa statistics showed overall agreement with the gold standard for these categories ($p<0.05$).

Conclusions: The agreement of our cytotechnologists in classifying AGUS as well as SIL is moderate with a tendency to overdiagnose AGUS and undercall SIL. While the moderate agreement may be due to their > 20 years work experience and constant feedback from the cytopathologist after cytology/histology correlation, more studies might be necessary to support our findings.

381 Efficacy of EUS-Guided Fine Needle Aspiration in the Diagnosis of Upper Gastrointestinal Lesions

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Background: Endoscopic ultrasonographic fine needle aspiration(EUS-FNA) has been utilized to evaluate submucosal lesions of the upper gastrointestinal(UGI) tract with reported sensitivities of 83-100%. Our study aims to examine the efficacy of UGI EUS-FNA in our institution, including the role of rapid on-site evaluation(ROSE).

Design: From 9/08 to 8/15, all UGI EUS-FNA cases in our database were analyzed.

Results: In our 7 year study, 544 patients underwent 709 EUS-FNA. Most were from the pancreas(63%) and intraabdominal lymph nodes(16%). 110(16%) were UGI specimens (stomach=89, duodenum=11,esophagus=10) from 94 patients (stomach=75, duodenum=10, esophagus=9) including 53(56%) males and 41(44%) females. Most were gastrointestinal stromal tumor(GIST) at 28%, followed by leiomyoma(LM), schwannoma(PNST), gastric adenocarcinoma(ACA), neuroendocrine tumor(NET), and pancreatic heterotopia(PH) at 18%, 5%, 4%, 3%, and 3%, respectively.

Specificity for UGI EUS-FNA was 100%, with overall sensitivity of 80%. Sensitivity was 100% for duodenal lesions, but were 88% and 78% for esophageal and gastric EUS-FNA, respectively.

Of 31 GIST, 27(87%) were diagnosed by EUS-FNA. 100% of NET, 80% LM, 75% ACA, 40% PNST, and 33% PH were also detected by EUS-FNA.[Table 1] All nondiagnostic EUS-FNA except one (esophageal) were located in the stomach. Neither specific gastric location nor number of FNA passes had any bearing on diagnostic outcome. EUS-FNA missed 4 of the smaller GISTs(avg 1.68 cm), as well as the smaller PNST(avg 2.0 cm) and PH(1-2 cm). Lesional size was not a factor in other nondiagnostic cases.

Overall, 56% of UGI EUS-FNA cases underwent ROSE. ROSE correlated well with EUS-FNA diagnosis in all ACA, PNST, PH, and 91% of GIST. Lack of ROSE was noted in nondiagnostic EUS-FNA cases in 2 of 4 leiomyomas, 1 of 3 PNST, 1 of 2 PH, as well as the only nondiagnostic ACA case.

Conclusions: EUS-FNA was effective in the diagnosis of UGI GIST, LM, ACA, and NET, but detected only 40% PNST and 1/3 PH. Gastric location and small size were associated with nondiagnostic results. The absence of ROSE was also a contributing factor for nondiagnostic LM, PNST, ACA, and PH EUS-FNAs.

	GIST#(%)	LM#(%)	PNST#(%)	ACA#(%)	NET#(%)	PH#(%)
Esophagus	1(10)	7(70)	0	0	0	0
Stomach	29(33)	13(15)	5(6)	4(4)	0	3(3)
Duodenum	1(9)	0	0	0	3(27)	0
Total UGI	31(28)	20(18)	5(5)	4(4)	3(3)	3(3)
ROSE%	71	50	80	75	100	67
Sens%	87	80	40	75	100	33
Avg size(cm)	3.3	2.4	2.7	3.1	4.5	1.8

382 Cytomorphologic Features and Immunoprofile Useful to Distinguish Clear Cell Papillary Renal Cell Carcinoma from Other Renal Cell Carcinomas in Cytology Specimen

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Background: Clear cell papillary renal cell carcinoma (CCPRCC) is an uncommon neoplasm of the kidney which has been recently defined. Although it shares some of the morhphologic features and immunohistochemical markers with clear cell renal cell carcinoma (CCRCC) and papillary renal cell carcinoma (PRCC), it is a distinct entity with a low grade indolent behavior and should be recognized on fine needle aspiration biopsy (FNA) or needle core biopsy (NCB).

Design: We retrieved all the FNA, NCB and surgical resection specimens of all RCC between 2011 and 2015. There were only six cases of CCPRCC with FNA smears and/or touch preparation of NCB (TP) which were confirmed on surgical resection. We selected 20 FNA/NCB cases of CCRCC and PRCC each to compare the cytomorphology and immunoprofiles.

Results: FNA smears or TP of CCPRCC showed tumor cells arranged in nests, papillary or 3-dimensional clusters, had moderate amount of delicate or clear cytoplasm with fine vacuoles, small eccentric nuclei with reverse polarity and inconspicuous nucleoli. There were a few scattered macrophages with pigments. In CCRCC FNA smears or TP, tumor cells were present in single, nests or sheets with transversing capillaries, had more abundant vacuolated cytoplasm, and had more central nuclei with prominent nucleoli. In PRCC FNA smears, tumor cells were present in papillary or 3-dimensional clusters, were columnar to cuboidal in shape, had scant to moderate granular cytoplasm, and eccentric nuclei possibly with prominent nucleoli and occasional longitudinal grooves. Foamy macrophages were seen in the background or within fibrovascular cores. Immunohistochemistry showed diffuse cytoplasmic positivity for CK7 and cup like membranous positivity for CA IX in CCPRCC. Tumor cells were less frequently and focally positive for CD10 and AMACR. CCRCC showed complete membranous positivity for CA IX and CD10, and negative staining for CK7 and AMACR. PRCC was positive for CK7 and AMACR, while was negative for CA IX.

Conclusions: Besides difference in histology and immunoprofiles among CCPRCC, CCRCC and PRCC, these entities can be distinguished on the basis of cytomorphologic features. In FNA, the key features to recognize CCPRCC are tumor cells arranged in nests, papillary or 3-dimensional clusters, moderate amount of delicate or clear cytoplasm with fine vacuoles, small eccentric nuclei with reverse polarity and inconspicuous nucleoli.

383 Informed Cytology and HPV Genotyping Preceding Biopsy Proven HSIL/Adenocarcinoma: Analysis from an Academic Medical Center

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Background: Cotesting (Pap and hrHPV) is currently the preferred cervical cancer screening method in the USA. At our institution we have an annual volume of 24,000 with 60% cotesting using the Roche cobas HPV test. While additional review of HPV+/NIL cases (informed cytology) is not mandated, we implemented re-screening of NILM/HPV+ Pap tests by a 2nd cytotechnologist (CT) in Sept 2013. For NIL/HPV+ cases, ASCCP guidelines suggest either repeat cotesting in 1 year or colposcopy for HPV 16/18 positive cases. In 2015, while we have no requests for primary HPV screening, we noted an increase in short term biopsy (bx) follow up after NIL/hrHPV cotest results.

Design: We retrieved all bx, excisions, and ECC diagnosed as HSIL and carcinoma between July 2014-June 2015 and preceding Paps done within a year of bx. All qualifying Paps and bx were reviewed by 2 pathologists. P16 IHC was performed on most CIN2 at diagnosis per LAST guidelines, and subsequently on all bx HSIL(CIN2) with preceding NIL/unsat Pap.

Results: 134 HSIL/Adenocarcinoma bx from 134 patients qualified for the study. HPV testing was not done in 53 cases (9 NIL/unsat, 44 ASCUS and higher); among the remaining were 2 HPV neg (1NIL, 1 LSIL), 57 HPV+ (ASCUS and higher) and 22 HPV+ (NIL/unsat). 19 CIN2/3, 2 AIS and 1 endocervical adenocarcinoma were preceded by NIL(21) or unsatisfactory(1) Paps. Concurrent hrHPV testing was done in all 21 bx with NIL Paps, and showed genotypes 16(10), 18(3), 16+18(1), 16+others(2), 18+others(1) and Others(4). All 21 NIL Paps had informed QC review by 2nd CT; additional pathologists review for this study was NIL(11), Unsat(1), ASCUS(2), ASC-H+ LSIL(1), ASC-H(1), HSIL(4) and AEC-favor neoplasia(1). The average bx time from abnormal Pap or positive hrHPV to bx was 42 days and did not differ significantly based on HPV genotype or Pap result. Review of bx/excision specimens for this study showed good concordance.

Conclusions: We found that informed CT Q/C review of hrHPV/NIL Paps can miss high grade abnormalities of bx proven CIN2/3 and carcinoma. Prompt colposcopic follow-up of NIL/hrHPV co-test results, particularly 16/18 genotypes, results in earlier detection of high grade squamous and glandular lesions; either unsampled or undetected on a concurrent Pap test. Considering clinician variability in screening practices and follow up of co-test results as well as our quality assurance, we have implemented additional pathologist review of NIL/HPV 16/18 positive Paps.

384 Morphologic Criteria for Atypia in Upper Urinary Tract Cytology: Should It Be Different from the Lower Urinary Tract Criteria of the Paris System of Reporting Urinary Tract Cytology (TPS)?

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Background: Upper urinary tract (UUT) cytology is difficult and innately "atypical". The Paris System of Reporting Urinary Cytology has defined distinct reporting categories and diagnostic criteria mostly studied in the lower tract urinary cytology. This study investigates if these specific criteria are applicable for diagnosing UUT cytology.

Design: Our institutional database from 1/1/2010 to 12/30/2014 was searched for UUT cytology cases with a follow up surgical biopsy of the UUT or nephroureterectomy within a year of the acquisition of cytology specimen. The cases were reviewed to evaluate the following criteria: Nuclear cytoplasmic (NC) ratio, nuclear hyperchromasia, irregular nuclear contours, coarse chromatin, prominent nucleoli, cell-in-cell appearance, pushed out nuclei, number of atypical cells, pseudopapillary structures, pleomorphism, anisonucleosis, mitosis, cytoplasmic tails, degenerative changes and cytoplasmic vacuolization. Receiver operator characteristic curves established the diagnostic value of each morphologic feature using an AUC cut off value of 0.5.

Results: A total of 55 UUT cytology cases were identified from 497 of UUT cytology specimens accessioned during this time. These cases include 17 (31 %) negative for high grade urothelial carcinoma (HGUC), 9 (16 %) as atypical urothelial cells, 6 (11 %) as suspicious for HGUC and 23 (42 %) as HGUC. The criteria most predictive for HGUC include irregular nuclear contours and number of atypical cells as the most frequently observed morphologic parameters in the "positive for HGUC on follow-up" group.

Criteria	Area under the curve (AUC)	95% CI lower bound	95% CI upper bound
Number of atypical cells	0.9143	0.85094	0.97763
Irregular nuclear membrane	0.7947	0.67903	0.91044
Cytoplasmic tails	0.7683	0.65462	0.88193
Pleomorphism	0.7478	0.62492	0.87070
Coarse chromatin	0.7286	0.64485	0.81229
Increased N/C ratio >0.5	0.6857	0.60451	0.76692
Cell-in-cell appearance	0.6797	0.55257	0.80682
Anisonucleosis	0.6301	0.51624	0.74399
Nuclear hyperchromasia	0.6165	0.52475	0.70833

Conclusions: Based on the morphological analysis we conclude that the criteria proposed in TPS are applicable to the UUT cytology. The most significant criteria in detecting HGUC are the increased number of atypical urothelial cells in addition to irregular nuclear contours, nuclear hyperchromasia, high NC ratio, and coarse chromatin.

385 Multiple Squamous Cells in Thyroid Fine Needle Aspiration: Friends or Foes?

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Background: Abundant squamous cells are rarely encountered in thyroid fine needle aspiration specimens with only few case reports noted in the literature. Their presence and cytologic features may pose a diagnostic dilemma and challenges for proper classification and clinical follow up. We intend to gain more insight into the frequency of this finding and its clinical significance

Design: Our 16 years electronic records were searched to reveal 15 thyroid fine needle aspirates with abundant squamous cells. The available cytology and surgical resection slides were reviewed and clinical follow up was documented.

Results: Only 15 out of 8811 thyroid fine needle aspirates from our department contained predominantly squamous cells (0.17%) of which 2 were interpreted as non-diagnostic, 2 as atypical, 7 as benign, 1 malignant and 3 cases received a descriptive interpretation mentioning that distinction between benign and malignant entities cannot be done with certainty. Surgical follow up was available in eight cases only, with benign lesions representing the majority of the cases (squamous metaplasia in Hashimoto thyroiditis, benign epidermoid cyst, thyroglosal duct cyst, and one case of moderately differentiated squamous cell carcinoma). The cases without surgical resection were stable on ulterior ultrasound studies.

Conclusions: Thyroid aspirates with predominance of squamous cells cannot be classified in the known Bethesda categories. Even when interpreted as atypical or equivocal, the squamous cells present in our small case series were mostly benign. The only malignant case was easily identified cytologically because of its higher degree of differentiation. The most common pitfall for atypical squamous cells in these aspirates was squamous metaplasia in the setting of Hashimoto thyroiditis.

386 Utility of Next Generation Sequencing (NGS) Cotesting to Recategorize Bethesda-I Thyroid FNAs

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Background: Thyroid fine-needle aspiration (FNA) is a rapid, cost-effective, safe, and reliable procedure for the initial evaluation of thyroid nodules. The Bethesda System for Reporting Thyroid Cytopathology includes 6 categories of which category I poses a particular problem and requires a repeat FNA. Meta-analysis studies show the rate of Bethesda-I thyroid FNAs ranging from 1.8% to 23.6% with an overall value of 12.9%. The risk of malignancy in this category is 1-4%. Co-testing NGS panels like ThyroSeq may allow further categorizing Bethesda-I thyroid FNAs and more accurate management.

Design: We reviewed thyroid FNAs performed in our institution diagnosed as Bethesda-I from 7/1/14-1/31/15 with no NGS co-testing (group 1), and compared them to thyroid FNAs diagnosed as Bethesda-I from 2/1/15-8/31/15 with accompanying NGS co-testing using ThyroSeq v2.1 panel which was obtained by an additional pass taken by the Endocrinologist dedicated for NGS assay (group 2). The rate of Bethesda I FNA diagnosis is 11% in group 1 (28/251) and 10.8% in group 2 (25/231).

Results: Group 1 consisted of 28 FNAs, of which 11 (40%) had repeat FNA (standard of care) to further triage management. Of these, 5 (45%) were diagnosed as non-diagnostic again (Bethesda I), 4 (36%) were reclassified as benign (Bethesda II), and 2 (18%) were reclassified as atypical (AUS) (Bethesda III).

Group 2 consisted of 25 FNAs, of these, 19 (76%) had ThyroSeq co-testing which allowed to reclassify as : probably benign with no mutations detected 10 (40%), atypical with abnormal mutation detected 2 (8%). A total number of 7 (28%) could not be tested with NGS due to insufficient cellular material in the dedicated sample. One of the cases with mutation detected was a high risk mutation (Nras). Among this group, 3 (12%) had repeat FNA.

Repeat rate in group 1 is noted as 40% whereas it is decreased to 12% in group 2,(p-value:0.024).

Conclusions: We believe the use of a dedicated pass for NGS co-testing panel in thyroid FNAs with a diagnosis of Bethesda-1 will help further triage these patients, reduce the repeat FNA rate, and decrease patient non-compliance rate.

387 Paris System for Reporting Urine Cytology, a First Approach

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Background: Urine cytology is a simple, inexpensive tool and a key element in the surveillance of patients with urothelial carcinoma (UC). The American Urological Association guidelines recommend the use of urinary cytology to screen and evaluate patients at high risk for UC and monitor recurrence, progression, or treatment response. The The International Academy of Cytology (IAC) and The American society of cytopathology (ASC) at the international cytology congress presented the Paris system (PS) for reporting urinary cytology. The system categories have been recently defined and include: I. Adequacy, II. Negative for high-grade urothelial carcinoma (NHGUC), III. Atypical urothelial cells (AUC), IV. Suspicious for high-grade UC (SHGUC), V. High grade UC (HGUC), VI. Low-grade urothelial neoplasm, VII. Other malignancies, primary and secondary.

Design: A retrospective review of urine cytology cases with concomitant biopsy from 2013 to 2015 at our institution was performed. The diagnoses were re-classified using the PS categories.

Results: Of 110 urine cytology cases, NHGUC (PSII) represented 4% (4/110), AUC (PSIII) 33% (37/110), SHGUC (PSIV) 28% (31/110), HGUC (PSV) 31% (34/110) and LGUC (PSVI) 4% (4/110). Malignancy rates are 60%, 97%, 85% for PSIII, IV and V. Only one PSVI case showed low-grade UC, the remaining had non-invasive (WHO 2/3) and invasive (WHO 3/3) HGUC.

Paris Category	%(# cases)	Malignancy Rate %
II	4(4)	0
III	33(37)	60
IV	28(31)	97
V	31(34)	85
VI	4(4)	75
Total	100 (110)	

Conclusions: Adoption of the Paris System offers standardization for urine cytology reporting which will improve the screening and surveillance of UC patients. PSII offers a better way to understand the report of the urine cytology for patient's management since it would be used to rule out HGUC. PSIII category may under-diagnose HGUC. PSVI may be controversial due to the challenge of diagnosing LGUC in urine cytology and the recently reported histological grading migration for UCs previously called WHO 2/3 and now classified as HGUC using architecture, cellular atypia and mitoses, which are not all part of the PS criteria. This system will have a learning curve for the cytopathologists. They will need to adapt to the new criteria and terminology.

388 Fine Needle Aspiration Biopsy Material of Liver Lesions Yields Higher Percentage of Tumor Cells for Molecular Studies: A Direct Comparison to Concurrent Core Biopsy

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Background: Historically, fine needle aspiration (FNA) samples have been considered to be inferior for molecular studies when compared to core biopsies because of differences in gauge of needles. However, as more and more molecular targets are discovered for potential cancer therapeutics, a tissue sampling modality that results in the most concentrated tumor samples without contaminating cells is essential.

Design: The primary goal of this study is to compare the tumor concentration of tissue samples from concurrent FNA and core biopsy samples in primary or metastatic liver lesions. Seventy patients with liver masses who underwent concurrent FNA and core biopsies at two medical institutions were included in this retrospective study. FNA and core biopsy material were evaluated for diagnostic suitability and tumor concentration.

Results: Diagnostic accuracy was 94% for FNA and 90% for core biopsy. Tumor cells were present in the cell block in 83% of samples versus 90% of core biopsies with FNA material yielding an average of 82% tumor cell purity in comparison to 35% in core biopsy material.

Conclusions: FNA material has increased tumor concentration compared to core biopsy and is, thus, more suitable for molecular studies. The high percent of tumor cells in FNA material is due to the preferential stripping of loosely adherent neoplastic cells leaving most of the stroma behind. In conclusion, FNA sampling should be considered when tumor cell purity is paramount. Furthermore, FNA carries less morbidity than core biopsy and in some situations core biopsy is not feasible due to risks associated with the sampling such as bleeding and damage to adjacent vital structures.

389 Cytopathologist-Performed Ultrasound-Guided Fine Needle Aspiration of PET-Detected Lesions

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Background: Positron emission tomography (PET) is a highly sensitive method for detecting metastatic involvement of non-palpable subcentimeter lymph nodes, but false-positives can occur due to inflammation or therapy-related changes. Cytopathologist-performed ultrasound-guided fine needle aspiration (US-FNA) enables sampling and confirmation of true disease with a reported specificity of 100%. In this study, we demonstrate the ability of pathologists to successfully sample these lesions and the impact of cytopathologist-performed US-FNA of PET-detected lesions following the introduction of US to our on-call FNA service.

Design: We queried our pathology database for cytopathologist-performed FNAs from January 2012 to March 2015, then identified those performed with US-guidance. For all cytopathologist-performed US-FNAs, we retrospectively reviewed the imaging modality used to initially detect the lesion, lesion size and location, and final pathologic diagnosis.

Results: Of the 2,178 cytopathologist-performed FNAs, 593 (27%) were US-FNA including 109 (5%) of PET-detected lesions (Table 1). Of the PET-detected cases, the majority (n=85, 78%) were lymph nodes (cervical, submandibular, supraclavicular, axillary, and inguinal) and the remainder were thyroid (n=13, 12%) and salivary gland (n=11, 10%). Example final pathologic diagnoses for lymph node, thyroid, and salivary gland cases included metastatic squamous cell carcinoma, papillary thyroid carcinoma, and Warthin's tumor, respectively. The average size of targeted lymph nodes and the rate of obtaining diagnostic material are demonstrated in Table 2.

Table 1, Volume of cytopathologist-performed US-FNA of PET-detected lesions.

Time frame	Total cytopathologist-performed FNA (n)	US-FNA (n)	PET-detected US-FNA (% of Total FNA)
2012 (Jan-Dec)	534	2	1 (<1%)
2013 (Jan-Dec)	624	157	35 (5.6%)
2014 (Jan-Dec)	806	336	52 (6.5%)
Jan-Mar 2015	214	98	21 (9.8%)

Table 2, PET-detected lymph nodes: average size and rate of adequate samples.

Time frame	PET-detected lymph nodes (n)	Average size (cm)	Diagnostic material obtained (%)
2012 (Jan-Dec)	0	N/A	N/A
2013 (Jan-Dec)	29	1.7	27/29 (93%)
2014 (Jan-Dec)	39	1.5	37/39 (95%)
Jan-Mar 2015	17	2.0	15/17 (88%)
TOTAL	**85**	**1.6**	**79/85 (93% overall)**

Conclusions: The increased use of PET imaging to evaluate for metastatic disease has led to a substantial increase in demand for FNA biopsies of these lesions. This study demonstrates that pathologists can accurately correlate PET findings with US images and successfully sample small, non-palpable lesions with US-FNA.

390 "Benign Endometrial Cells" Reporting on Pap Smears (The 2014 Bethesda System): Is ≥45-Year Cutoff Appropriate? A Study Based on 672,000 Pap Smears with Follow-Up

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Background: The new 2014 Bethesda System for reporting cervical cytology recommends that benign endometrial cells (BEC) should be reported only in patients ≥45 years. This is a change from the previous wherein BEC were reported in patients ≥40 years. Small population studies have shown that in patients ≥40 years with BEC, there is an approximately 1% chance of an abnormal finding in further work-up. The current study is designed to test whether a higher age threshold change will increase the specificity of the test to predict significant endometrial pathology.

Design: 1177 Pap smears reported as BECs from 01/01/2007 to 12/31/2014 in patients aged ≥40 years were retrieved after Institutional Review Board (IRB) approval from our archival files of 672,000 ThinPrep Pap smears. The results of subsequent work-up (e.g. endometrial biopsy) were collected, significant findings documented and Two-tailed Fisher Excat Probability Test was done to compare patient groups <50 and ≥50 years.

Results: The dataset was sub-divided by patient age in groups with follow-up results presented in the following table.

Patient's Age (Years)	40-44	45-49	50-54	55-59	>60
Number of BEC	308	454	295	74	46
Number of biopsies	131	210	158	39	23
Atypical endometrial hyperplasia (%)	0	2 (1.0%)	1 (0.6%)	0	2 (8.7%)
Endometrial cancer (%)	0	1 (0.5%)*	5 (3.2%)	0	4 (17.4%)
Total (%) abnormal	0	1.5%	3.8%	0	26.1%
Total (%) abnormal (combined age groups)	0.9%		5.5%		

*Adenosarcoma on concurrent biopsy.

The overall incidence of endometrial carcinoma or hyperplasia, in all patient groups combined, is 2.7% on follow-up biopsies. However, in age groups <50 the incidence is 0.9% with only one case of true malignancy, an adenosarcoma, while the incidence is 5.5% in age groups ≥50 (p=< 0.001).

Conclusions: Our study suggests that BECs are useful in screening for endometrial leisons (carcinoma/hyperplasia) in patients ≥50 with 5.5% of cases having histologic confirmation of significant endometrial disease. In contrast, for patients <50 there was only one malignancy and two endometrial hyperplasias confirmed on follow-up biopsies (incidence of 0.9%). These data support raising the age threshold to ≥50 to increase the specificity of the test when reporting endometrial cells on Pap smears, while emphasizing the importance of clinical correlation with patient's menstrual status and other relevant symptomology.

391 Comparison of GATA-3, GCDFP-15 and Mammaglobin Expression in Breast Carcinoma in Serous Effusions: A Cell Block Microarray Study

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Background: GATA-3 is a transcription factor involved in the differentiation of human tissues. It has proven to be a successful marker for breast and urothelial carcinoma. Immunohistochemical data show that it is more sensitive than Mammaglobin (MAM) and GCDFP-15 in diagnosing metastatic breast cancer. Using cell-block Micro-Arrays (TMA), we aim to compare GATA3 to GCDFP-15 and MAM in the work up of malignant effusions and the diagnosis of breast carcinoma in fluids.

Design: Following IRB approval the laboratory archives were searched for cell blocks from malignant effusions with confirmed breast cancer diagnosis. A total of 27 breast cancer cases with adequate cell block cellularity were selected. Cell block Micro-Arrays (TMA) were constructed with 3 cores from each cell block using a 1 mm core needle. Microarray slides were stained for GATA-3, GCDFP-15, MAM, ER and PR. Only nuclear stain was considered positive for GATA-3, ER and PR and cytoplasmic stain for MAM and GCDPF-15. The intensity and percentage of reactive cells were recorded. Results were considered positive if greater than 5% of cells showed expression.

Results: All cases were well represented in the TMA sections. GATA-3 showed nuclear expression in 85% (23/27) of cases, ER was positive in 70% (19/27) of cases, PR was positive in 55% (15/27) of cases, MAM showed cytoplasmic expression in 52% (14/87) of cases while GCDFP-15 showed cytoplasmic expression in 37% (10/27) of cases. All MAM and GCDFP-15 positive cases expressed GATA-3 while MAM and GCDPF-15 co-stained only 9 cases. GATA-3 stained 50% or more of tumor cells in 87% (20/23) of positive cases compared to MAM 78% (11/14), and GCDPF-15 60% (6/10). Of the 19 ER positive cases 95% (18/19) were positive for GATA-3 compared to 58% (11/19) for MAM and 34% (6/19) for GCDFP-15.

Marker	GATA-3	MAM	GCDFP-15	ER	PR
GATA-3	**23**	14	10	18	14
MAM	14	**14**	9	11	7
GCDFP-15	10	9	**10**	6	5
ER	18	11	6	**19**	14
PR	14	7	5	14	**15**

Conclusions: When compared to MAM and GCDFP-15, GATA-3 shows greater sensitivity for the diagnosis of breast cancer in serous effusion. Unlike GATA-3, MAM and GCDFP-15 expression appears to be unrelated to ER status.

392 Factors Impacting the Performance Characteristics of Bile Duct Brushings (BDBs); an Analysis in 444 Patients

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Background: Literature on factors affecting the performance characteristics of BDB is limited.

Design: Data from index BDBs of 444 patients over 15 yrs at a combined primary/tertiary care center with >7 cytopathologists serving multiple gastroenterology groups was analyzed.

Results: Cytology diagnoses (Dx) were non-Dx 11(2.5%);negative 284(64%);atypical 62(13.9%);suspicious 34(7.7%) and malignant 53(11.9%); 253(57%) had followup (FU) including 135 histologically confirmed cancers, 22 cancer related deaths and 96 benign (≥18 mos of uneventful FU).

Table 1. Factors Affecting BDB Performance in Cases with Known FU(n=253)

Cytology Dx (n)	**%**	**Sensitivity %**	**Specificity %**	**PPV%**	**NPV%**	**Accuracy %**
Malignant (35)	14	35	100	100		66
Suspicious (22)	9	24	95	95		63
Atypical (37)	15	35	98	95	58	65
Malignant+ suspicious+ atypical (94)	37	58	97	97		73
Preparation Method (n)						
Smears (29)	18	67			74	83
Smears+Cytospin (29)	18	50	100	100	55	69
ThinPrep(TP)(60)	38	51			45	65
TP+cell block (41)	26	61	80	91	40	66
Stent Status (n)						
Stent (43)	17	47	100	100	74	79
No stent (210)	83	59	96	97	54	71

Table 2. Overall Risk of Malignant Outcome Based on BDB Result

Cytology Dx	**Benign Outcome**	**Malignant Outcome**	**RR**	**95% C.I.**	**p value**
Benign	93	66	1.00	-	-
Atypical	2	35	2.28	1.87,2.78	
Suspicious	1	21	2.30	1.87,2.83	<0.0001
Malignant	0	35	2.41	2.00,2.90	

Type of carcinoma (cholangio vs pancreatic ductal) had no effect on accuracy of final Dx (p 0.603) or BDB classification (p 0.844), nor did time of performance (first 7.5 vs latter 7.5 yrs)(p 0.131).

Conclusions: While BDB sensitivity was fairly low in identifying malignancy (1/3 cancers correctly identified), specificity and PPV were high(0 false positives). When atypical, suspicious and malignant Dx categories were combined, specificity and PPV dropped minimally(to 97%) but sensitivity almost doubled(58%). Presumably because of operator familiarity, smears trended toward a higher accuracy rate than ThinPreps(p 0.084). The higher specificity in stented vs nonstented patients suggests that cytopathologists only call such cases malignant when evidence is overwhelmingly convincing. Cancer type and time frame performed did not impact performance. Better morphologic criteria are needed to improve overall performance of BDB.

393 When We Say "Maybe", We Mean "Yes"; We Just Don't Want to Say It (and Probably for a Good Reason): Pathologists' Own View on Their "Uncertain" Diagnosis in Bile Duct Brushings

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Background: Uncertain diagnoses are rendered commonly in bile duct brushing (BDB) specimens. Diagnostic terms of "atypia" and "suspicious" are used and interpreted highly variably by the operators.

Design: 7 reviewers with different experience levels, all involved in daily pathology practice of bile duct brushings, were tasked to blindly review 60 BDBs (30 of which were confirmed as malignancy by F/U resection, and the other 30 as benign by either resection or ≥18 mo uneventful f/u), and diagnose them as benign (1), atypical (2), suspicious (3), and malignant (4). For the atypia group, three options of reactive (2A), NOS (2B) and "cannot exclude carcinoma" (2C), were provided. And finally, for each case, the reviewers were forced to commit to either benign or malignant diagnosis (**forced diagnosis**).

Results: The results are summarized in table 1. These figures did not seem to vary by experience.

First diagnosis	Forced diagnosis*	Benign outcome	Malignant outcome
2A (atypical, favor reactive)	B:100%	69%	31%
	M:0%		
2B (atypical, not otherwise specified)	B:95%	66%	34%
	M:5%	50%	50%
2C (atypical, cannot exclude carcinoma)	B:11%	50%	50%
	M:89%	23%	77%
3 (suspicious)	B:0%		
	M:100%	18%	82%
B:benign, M:malignant			
*Forced diagnosis: When reviewers were forced to choose either benign or malignant.			

Conclusions: When forced, pathologists classify the vast majority of the "atypical/cannot rule out carcinoma" or "suspicious" cases as "malignant" (89 and 100%, respectively) but 23 and 18% of these, respectively, in fact prove to be benign (would be over-calls) by follow up. Therefore, these 2 uncertain categories remain useful to avoid false-positives. It should be conveyed to the management teams that with these 2 diagnostic categories the pathologists actually mostly mean malignancy, however, it should also be kept in mind that a not too insignificant percentage of them will prove to be benign.

394 Differential Expression of Various Clones of Estrogen Receptor in Cell Block Preparation of Lung Adenocarcinoma

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Background: Women treated for breast cancer sometimes develop pulmonary lesions that may represent either a metastasis or a new primary tumor, most commonly an adenocarcinoma. The differential diagnosis is particularly important in initial samples of the lung lesion obtained by less invasive procedures, such as fine needle aspiration (FNA) or a pleural effusion (PE). Estrogen receptor (ER) positivity has generally been used to favor breast over lung primary. However, recent studies have shown variable results for ER expression in lung adenocarcinoma, ranging from 0 to 80%. Therefore we studied the frequency of ER expression in cytology samples of lung adenocarcinomas with three different monoclonal antibodies.

Design: Cytology cell block preparations from 41 patients (22 lung FNAs and 19 PEs) with clinically and histologically documented primary lung adenocarcinoma were initially stained with commercially available monoclonal antibodies to two different ER clones; clone 6F11 (Vector Laboratories, Burlington, CA) and clone 1D5 (DAKO, Carpinteria, CA). In 29 cases, sufficient material remained in the cell block to stain with a third antibody to ER clone SP1 (Cell Marque, Rocklin, CA). The extent of ER nuclear staining was scored as 3+ (50-100% of tumor cells), 2+ (11-50%), and 1+ (up to 10%).

Results: Positive immunostaining for ER-6F11 clone was observed in 4 of 22 lung FNAs (18.2%, 2+). Two of the four ER-6F11 positive FNAs also stained with ER-1D5 clone (9.1%, 2+). None of the 15 FNAs or 14 PEs showed immunoreactivity for ER-SP1. No immunoreactivity for ER was observed with any of the clones in any of the 19 malignant PEs.

Conclusions: A small subset of pulmonary adenocarcinomas show positivity for ER clones 6F11 and ID5 in FNA samples (18.2% and 9.1%, respectively). The absence of immunoreactivity for ER-SP1 clone may suggest a higher specificity of this clone in lung adenocarcinoma in our study. These results suggest that while diagnostic caution should be exercised when using 6F11 and 1D5 clones of ER in the differential diagnosis with breast carcinoma, the ER-SP1 clone appears to be more specific when applied to lung tissue. Further larger studies are needed to support this observation. Meanwhile, the differential diagnostic value of all clones of ER in malignant PEs appears to be secure.

395 Clinical Outcome of "LSIL Cannot Exclude HSIL" Supports the Recommendation of "LSIL and ASC-H" in the 2014 Bethesda System

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Background: The term low-grade squamous intraepithelial lesion (LSIL) cannot excluded high-grade squamous intraepithelial lesion (LSIL-H) has been suggested for cases of predominant cell type in favor of LSIL but also with a few immature cells showing increased nucleus to cytoplasmic ratio and atypical nuclear features, which are insufficient for the diagnosis of high-grade squamous intraepithelial lesion (HSIL). In the 2014 Bethesda System for Reporting Cervical Cytology, the use of LSIL-H is discouraged as a diagnostic category as it may lead to an intermediate category against the concept of two-tier classification. Instead, it recommends using two categories, LSIL and atypical squamous cells cannot exclude HSIL (ASC-H), in such occasion. The aim of this study was to investigate the outcome of LSIL-H in compares to other squamous cell abnormalities and to determine if the recommendation was justified.

Design: A total of 134198 consecutive Papanicolaou tests using conventional smear in the period of 2010 to 2014 were retrieved from the computer database. All the categories of squamous cell abnormalities were analyzed for their diagnostic rate, biopsy rate, and paired histologic follow-up.

Results: LSIL-H was rarely used as a diagnostic category in our institute (n=23, 0.02%). Follow-up biopsy was performed on 19 patients (82.6%) and showed LSIL in 6 patients (31.6%) and HSIL or more severe lesions (HSIL+) in 10 patients (52.6%). The associated LSIL rate was significantly higher than ASC-H (4.6%, p=0.001) but similar to LSIL (41.5%), while the associated HSIL+ rate was significantly higher than LSIL (10.9%, p<0.001) but similar to ASC-H (50.0%).

Cytologic Interpretation	N (% all pap smears)	No. of Biopsy (% N)	Follow-Up Histologic Results (% No. of Biopsy)		
			Negative	LSIL	HSIL+
ASC	693 (0.52)	470 (67.8)	327 (69.6)	75 (15.9)	68 (14.5)
LSIL	358 (0.27)	248 (69.3)	118 (47.6)	103 (41.5)	27 (10.9)
ASC-H	146 (0.11)	130 (89.0)	59 (45.4)	6 (4.6)	65 (50.0)
LSIL-H	23 (0.02)	19 (82.6)	3 (15.8)	6 (31.6)	10 (52.6)
HSIL	410 (0.31)	373 (91)	56 (15.0)	10 (2.7)	307 (82.3)

Conclusions: Given the rarity of using LSIL-H and the comparable follow-up LSIL rate as LSIL and follow-up HSIL rate as ASC-H, our data supports using "LSIL and ASC-H" as recommended by the 2014 Bethesda System. The patients should be managed as ASC-H due to the high associated HSIL rate in follow-up.

396 TROP-2 and CD117 Expression in Oncocytic Lesions of the Thyroid: Exceptional Sensitivity and Mediocre Specificity for Oncocytic Papillary Thyroid Carcinoma

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Background: The presence of Hurthle cells in thyroid fine needle aspirates (FNAs) presents a challenging differential diagnosis. TROP-2 and CD117 have recently been introduced as being highly specific and sensitive for papillary thyroid carcinoma (PTC), but no studies have evaluated their diagnostic utility in oncocytic PTC compared to benign oncocytic lesions. Our aim was to investigate the sensitivity and specificity of TROP-2 and CD117 immunohistochemistry (IHC) for oncocytic PTC.

Design: A retrospective search of thyroid resections from 1996 to present revealed 17 PTC cases with oncocytic diagnostic phrasing and 62 benign lesions with oncocytic change, including: 23 cases of nodular hyperplasia, 21 cases of lymphocytic thyroiditis, and 18 cases of follicular adenoma with Hurthle cell change. A tissue microarray was constructed with 6 cores from each case and IHC for TROP-2 and CD117 was performed. IHC for CD117 was evaluated for membranous and/or cytoplasmic staining and graded as negative or positive (staining in ≥10%). IHC for TROP-2 was evaluated for membranous staining and graded as negative or positive (staining in ≥25%). On H&E, PTC cores were subclassified as having oncocytic features or diffuse oncocytic change (PTCONC). In benign cores, oncocytic change was classified as normal with oncocytic features, Hurthle cells (H), Hurthle cells with atypia (Hatyp), or lymphocytic thyroiditis with oncocytic features/Hurthle cells (LTH).

Results: Lack of CD117 staining showed 70.5% specificity and 98.6% sensitivity for malignancy (243 cores). Only one core of one case of PTCONC showed weak patchy CD117 staining in 50-75% of tumor cells. TROP-2 staining showed 43.9% specificity and 100% sensitivity for malignancy (249 cores). No core from oncocytic PTC was negative for TROP-2, with none showed <50% moderate membranous staining. The specificities of CD117 loss and TROP-2 positivity were limited by downregulation of CD117 and abberant TROP-2 expression in cells with exuberant Hurthle change (H, Hatyp, LTH).

Conclusions: Our results show that the sensitivity of CD117 and TROP-2 for classic PTC extends to the evaluation of oncocytic PTC. However, previously reported specificity as high as 89% for TROP-2 and 100% for CD117 do not apply to the oncocytic differential diagnosis. Downregulation of CD117 and abberant expression of TROP-2 is commonly seen in benign Hurthle change and represents a novel finding. These pitfalls are crucial to bear in mind if these antibodies are applied to specimens with limited cellularity such as FNAs.

397 The Paris System for Reporting Urine Cytology (PSRUC) Improves the Prognostic Value of an 'Atypical Urothelial Cell' Diagnosis

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Background: We sought to investigate whether using the PSRUC improves the accuracy of different cytological categories in relation to histological outcome, in comparison to the currently used system in our laboratory

Design: 124 lower urinary tract cytological specimens with histological correlation within 1 year follow-up were randomly selected. Cytological slides were blindly reviewed and assigned to one of the PSRUC categories: benign, atypical urothelial cells (AUC), suspicious for high-grade urothelial carcinoma (S), and positive for high-grade urothelial carcinoma (P). Original cytological diagnoses as well as histological outcome (benign, low-grade UC, HGUC) were also recorded. Original and reviewed cytological diagnoses were correlated with a subsequent histological diagnosis of HGUC.

Results: Complete results are shown in [table 1]. While significantly fewer cases were given an AUC diagnosis using the PSRUC in comparison to the original diagnoses (26% versus 39%), the rate of S+P remained unchanged (≈38%) in the cohort. Using the previous system, 35% of the AUC cases had a histological follow-up of LGUC in comparison to 26% of the benign category. Applying the criteria of the PSRUC resulted in a higher number of histologically confirmed LGUC to be assigned to the benign category (40%) rather than AUC (22%). The performance of an S/HGUC diagnoses did not change using the two systems and the predictive value for subsequent HGUC was 94%. Importantly, the association of an AUC diagnosis with subsequent HGUC significantly increased using the PSRUC in comparison to the original AUC category (predictive value of 53% versus 33%, respectively. p=0.045).

Cytology (PSRUC)	Benign (n=45; 36%)	AUC (n=32; 26%)	Suspicious (n=18; 15%)	Positive (n=28; 23%)
Histology				
Benign	19	8	3	0
Low-Grade	18	7	0	0
High-Grade	8	17	15	28
PV for HG	*18%*	*53%*	*83%*	*100%*
Cytology (Original)	**Benign (n=27; 22%)**	**AUC (n=48; 39%)**	**Suspicious (n=23; 18%)**	**Positive (n=26; 21%)**
Histology				
Benign	13	15	1	1
Low-Grade	7	17	1	0
High-Grade	7	16	21	25
PV for HG	*26%*	*33%*	*91%*	*96%*

Conclusions: The three impacts of applying the PSRUC in our cohort were: 1- a significant decrease in the number of cases diagnosed as AUC; 2-a significantly improved predictive value of the AUC category for a diagnosis of HGUC and 3-lower detection rates for LGUC on cytology.

398 Role of HPV Testing in Atypical Glandular Cells on Pap Smear Diagnosis

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Background: The diagnosis of atypical glandular cells (AGC) on pap smear is associated with underlying histological abnormalities such as reactive changes, cervical intraepithelial neoplasia (CIN) or/and glandular cells abnormalities of the cervix and/or the endometrium. The aim of our study was to determine the impact of HPV status and age in predicting the outcome of AGC diagnosis in our institution.

Design: A retrospective study from our pathology database of cases of AGC from 2009 to 2012. Glandular abnormalities were interpreted as per the Bethesda system Guidelines. HPV testing was performed by hybrid capture II. Only cases with follow-up and HPV testing were included. On follow-up, the outcome was considered non-significant if benign or CIN I, and significant if CIN II/III, adenocarcinoma in situ (AIS), squamous cell carcinoma (SCC), or endocervical carcinoma. Sensitivity, specificity and positive and negative predictive value (PPV and NPV) were calculated after omitting the endometrial carcinoma cases.

Results: A total of 318,583 pap smears were screened during the 4-year period. 107 cases of AGC diagnosis had concurrent HPV test and subsequent follow-up. Of the 107 cases: 70 (65%) were HPV negative and 37 (35%) were HPV positive. of the HPV negative cases: 54 (77%) showed non-significant outcome on follow-up, and 9 (12%) had significant outcome. Of 37 HPV positive:13 (35%) had non- significant outcome, and 24 (65%) showed significant outcome. The overall sensitivity and specificity was 72% and 79%, respectively. The overall PPV, and NPV was 62%, and 86%, respectively. In women ≤30 years of age the sensitivity of HPV testing was 87% and specificity was 54% compared to 67% sensitivity and 85% specificity in women >30. PPV was 54% in women ≤30 and 67% in women >30. NPV was 87% in women ≤30 and 85% in women >30.

		HPV - N(%)	HPV+ N(%)
Non-Significant outcome	Benign	49 (70)	10 (27)
	CIN I	5 (7)	3 (8)
Significant outcome	CINII/III	6 (9)	17 (46)
	AIS	1 (1)	5 (14)
	SCC	1 (1)	1 (3)
	Endocervical carcinoma	1 (1)	1 (3)
	Endometrial Carcinoma	7 (10)	0
Total		70 (65)	37 (35)

Conclusions: In our study, the usefulness of HPV testing in predicting glandular abnormalities is still debatable with an overall sensitivity of 72% and NPV of 86%. HPV testing is a useful ancillary tool when used in conjunction to pap test in predicting glandular cells abnormalities. Larger studies are still needed to evaluate the role of HPV testing in AGC pap diagnosis.

399 Stratified Mucin-Producing Intraepithelial Lesions of the Cervix: A Diagnostic Challenge in Cytopathology

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Background: Stratified mucin-producing intraepithelial lesion (SMILE) is a variant pattern of high-grade intraepithelial lesion displaying a hybrid of features of high-grade squamous intraepithelial lesion (HSIL) and endocervical adenocarcinoma in situ (AIS). It is classified as adenosquamous carcinoma in situ or the stratified variant of AIS. Studies on the cytomophologic features of SMILE are limited.

Design: We performed a retrospective (2008-2015) review of PAP tests obtained from 5 patients with concurrent hrHPV testing (Hybrid Capture 2, Digene) and histologic confirmation of SMILE. Cytomorphologic characteristics were reviewed and correlated with subsequent biopsies.

Results: A total of 5 cases of SMILE were identified. Mean patient age was 30 years (range 23-39 years). Initial cytologic diagnosis were ASC-H (n=2), ASCUS (n=1), AGC favor neoplastic (n=1), and HSIL with glandular involvement (n=1). On re-review, AGC, favor neoplastic was present in all cases in addition to the previous cytologic diagnosis. Interestingly, only 3 of 5 cases were positive for hrHPV. The salient cytomorphologic characteristics included large fragments of crowded, overlapping dysplastic cells with mucinous or abundant clear cytoplasm and enlarged nuclei with hyperchromasia and coarse chromatin. The architecture of atypical mucinous cells with stratification and perpendicular orientation of the basal nuclei at the edge of fragments were clues to support AGC, favor neoplastic in contrast to HSIL with glandular involvement . Histologic confirmation of SMILE as the dominant lesion was present in 4 cases; one case showed HSIL with glandular involvement as the dominant lesion with foci of SMILE.

Conclusions: We report the largest series of SMILE on cytopathology and illustrate the morphologic features. SMILE should be considered in the cytologic differential diagnosis of atypical glandular cells. The prognostic significant of SMILE is not known, however recent studies suggest that SMILE should be regarded as a variant of AIS for patient management purposes.

400 Stromal Tissue as an Adjunct Tool in the Diagnosis of Follicular Thyroid Lesions by Fine Needle Aspiration Biopsy

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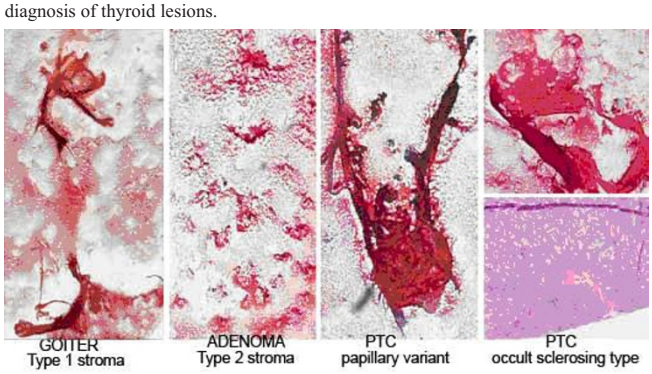
Background: Stroma from thyroid lesions acquired in fine needle aspiration biopsies (FNAB) has not been adequately evaluated.

Design: 256 consecutive cases of thyroid FNAB with follow-up were assessed. The stroma was categorized into three categories. Type 1a consisted of long, broad bands composed of mesh containing collagen fibrils thickened by entrapped blood components. Type 1b consisted of dense strands/bands with varying degree of hyalinization or fibroblastic proliferation; blood components were attached to the surface rather than being entrapped. Type 2 was similar to type 1a but with shorter and looser mesh containing collagen fibrils.

Results: Type 1a/1b stroma showed curved branching patterns suggestive of incomplete frameworks of nodular/papillary architecture or fragmented capsule. Within the nodular framework, delicate fibrous septae were also evident. Type 1b stroma likely represented thick fibrous septae or tumor capsules with reactive or sclerosing/hyalinizing changes. Examination of resected specimens showed that both Type 1a/1b were frequently associated with multinodular goiters (MNG) which are often hypocellular (61/72), or acellular (hence "inadequate for cytologic assessment", 6/72), and follicular neoplasms/papillary thyroid carcinoma (PTC) with moderate to marked cellularity (50/143). Type 2 stromal bands were scattered in the smears or confluent as diffuse sheets were associated with micro-follicles in encapsulated neoplasms (52/70) or with macro-follicles in MNG. Absent/inadequate stromal tissue was encountered in cases with large cysts and follicular neoplasms with oncocytic features/hypercellularity (4/70) and PTC with sclerotic stroma (16/73). In two cases, small nodules of occult sclerosing PTC was characterized by Type 1a/1b stroma and radiating stromal strands. Follicular lesions of undetermined significance (FLUS)(n=41) determined to be either negative (n=26) or positive (n=15) for carcinoma in follow up, was frequently associated with stromal characteristics of MNG and neoplasm respectively.

Conclusions: The preservation of the in vivo architecture in type 1a/1b stroma is likely due to elasticity. Recognition of the stromal architecture will likely facilitate the diagnosis of thyroid lesions.



401 Fine-Needle Aspiration of Neck Masses/Lymph Nodes Yields Adequate Material for HPV Testing in Head and Neck Squamous Cell Carcinomas

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Background: High risk-human papillomavirus (HR-HPV)-associated head and neck squamous cell carcinoma (HNSCC) is a distinct epidemiologic and pathologic disease that is increasing in frequency. Currently, there is no clinical standard for obtaining diagnostic material or determining HPV status in the workup of these patients. The purpose of this study was to determine the specimen adequacy for HR-HPV testing on material obtained by fine-needle aspiration (FNA) of neck masses and lymph nodes in patients with HNSCC.

Design: Cases were reviewed for patients who (1) underwent fine needle aspiration of neck masses/lymph nodes, (2) were diagnosed with HNSCC from July 2009 to June 2015, and (3) were tested for p16 immunohistochemistry (IHC) and HR HPV in situ hybridization (ISH).
Results: Of the 92 cases studied, the overall adequacy rate of cell block material was 87% (80/92) for HR-HPV ISH, 93% (86/92) for p16 IHC, 79% (73/92) for both and 96% (88/92) for either one. Of the 73 cases with both p16 IHC and HR-HPV ISH results, the overall concordant rate was 78% (57/73). 16 cases showed discordant results between p16 IHC and HR-HPV ISH, including 15 cases with positive p16 and negative HPV ISH, and 1 case with negative p16 and positive HR-HPV ISH.

Table 1: Cases with both p16 IHC and HR-HPV ISH results.

	IHC p16+	IHC p16-	Total
HR-HPV +	41	1	42
HR-HPV -	15	16	31
Total	56	17	73

Conclusions: FNAs of neck masses/lymph nodes yield adequate material for reliable HPV-related testing in HNSCCs and should be incorporated into the routine pathologic evaluation for these patients. Our data also showed an excellent concordance between p16 IHC and HR-HPV ISH, indicating p16 IHC may serve as a surrogate for HR-HPV infection in HNSCCs.

402 Fine Needle Aspiration Cytology of Histiocytic Sarcoma with Correlation of Histopathology and Immunohistochemistry

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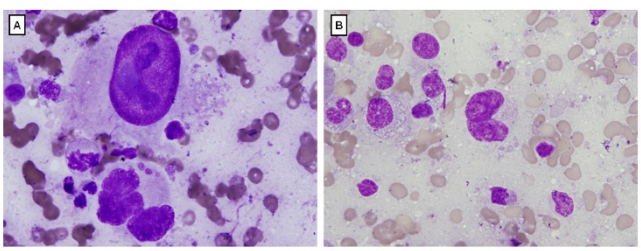
Background: Histiocytic sarcoma (HS) is a rare malignant neoplasm showing morphologic and immunophenotypic features of histiocytes. Patients with HS that present with extranodal masses often undergo diagnostic fine needle aspiration (FNA). However, FNA cytomorphologic features of HS have not been well described to date.
Design: A case series of HS with FNA cytology from 6 patients (8 samples) were reviewed along with histopathologic and clinical data. Cytologic features of HS in 5 published case reports were also reviewed.
Results: Of the 6 patients (4 men and 2 women, age range: 52-86 yr), 3 had a prior history of hematologic malignancy, 3 had a prior diagnosis of HS, and 4 died of disease in 4-19 months after diagnosis. FNA locations of the 6 primary and 2 metastatic/recurrent sites included neck, scapula, retroperitoneum, lung, and liver. Cytomorphologic features of HS included variably cellular smears, composed of predominantly dispersed epithelioid cells with reniform nuclei and irregular nuclear contour in all cases. All cases demonstrated pleomorphism, with subsets showing multinucleated "monster" cells (Fig. 1A), "PAC-man"-like histiocytoid cells (Fig. 1B), and single large nuclei that are >20 times larger than a red blood cell. Vesicular chromatin and conspicuous nucleoli were common. A lymphoplasmacytic or neutrophilic infiltrate was present, along with occasional emperipolesis and lymphoglandular bodies. Histologically, all HS cases showed sheets of large atypical histiocytes in an inflammatory background. Immunohistochemical positivity for CD68, CD163, and/or PU-1 was commonly observed. For the diagnosis of HS, the number of immunostains required ranged from 5 in recurrent cases to 20 in primary diagnoses on average.



Conclusions: Diagnosing HS by FNA cytology alone is challenging, given its rarity and the need for extensive work-up to exclude mimics including lymphomas, melanomas, carcinomas, and pleomorphic sarcomas. Nonetheless, FNA is a useful tool to document HS recurrence. Pleomorphic and epithelioid tumor cells with pronounced multi-nucleation and reniform or "PAC-man"-like histiocytoid cells in an inflammatory background should suggest the possibility of HS and judicious use of histiocytic lineage markers.

403 Cytologic Preparations for Next Generation Sequencing: An Analysis of Quality Indicators

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Background: Molecular testing is a routine part of the workup of many oncologic diseases and increasingly takes the form of targeted next generation sequencing (NGS). In clinical practice, many small biopsies fail testing due to low overall DNA concentration or tumor percentage. Current guidelines recommend core biopsies (BX) or cell blocks (CB) over cytology smears and liquid-based preparations (together called "CYT") because use of the latter sacrifices a diagnostic slide. CYTs, however, often contain abundant tumor cells and thus may provide adequate material for molecular testing when other materials are insufficient. In this study, we examine the performance of CYTs on a clinical NGS assay and recommend a workflow that incorporates use of these materials, with significant implications for patient care.
Design: Consecutive CYT, BX, and CB slides were reviewed for adequacy and tumor content by a molecular pathologist and/or cytopathologist. CYT slides were used when CB or BX were deemed inadequate; selected CYT slides were scanned and digital images stored for future reference. Tissue was scraped from the selected CYT slides or from 10 x 5 micron unstained slides for CB and BX cases. DNA was isolated per standard protocols. Sequencing was carried out using a hybrid capture NGS assay interrogating 309 cancer-related genes.
Results: The study includes 9 CYT, including lung and pancreatic adenocarcinoma, and carcinoma of unknown primary (CUP). 30 CB and 87 BX cases were used as comparitors. Median DNA concentration was 6.39 ng/ul for CYT, 10.45 ng/ul for CB, and 8.97 ng/ul for BX. 8/9 (89%) CYTs were successfully sequenced as compared to 29/30 (97%) CB and 79/89 (89%) BX. Median "mean target coverage" was 189.1 CYT, 147.8 for CB, and 156 for BX. The median percentage of loci with >30X coverage was 97.8% for CYT, 97.9% for CB, and 97.9% for BX. 4 of 8 (50%) of CYT had variants with implications for clinical trial enrollment, including lung tumors with *KRAS* mutation and *TSC2* loss of function, and a lung carcinoma and CUP with hypermutation.
Conclusions: Smears and liquid-based preparations generate DNA sequencing data of comparable quality and quantity to that obtained from core biopsies or cell blocks. By integrating digital scanning of cytology preps into the molecular workflow, these specimen types can serve as a valuable source of material for molecular testing, thereby avoiding additional biopsies for this purpose.

404 Fine-Needle Aspiration Cytology of Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features (NIFT), Single Institutional Study with Comparison to Invasive Follicular Variant of Papillary Thyroid Carcinoma

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Background: Noninvasive encapsulated follicular variant of papillary thyroid carcinoma (FVPTC) represents a distinct subset of papillary thyroid carcinoma with indolent behavior that can be managed clinically as a follicular adenoma. Therefore, the Endocrine Pathology Society Working group on encapsulated FVPTC has proposed that these lesions be re-categorized as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFT).
Design: A computerized search of our pathology laboratory information system was performed to identify all surgical pathology diagnosed as FVPTC with correlating thyroid fine needle aspiration (FNA) at our institution for the 5-year period from 2010 through 2014. Cases were divided into two groups: cases that meet the criteria for NIFT and cases with capsular or lymphovascular invasion. Cytologic diagnoses were compared.
Results: A total of 287 surgical pathology cases were identified, of which 65 had correlating FNA samples. 46 cases met the criteria for NIFT. Of the NIFT cases, 6 (13%) were diagnosed as benign (B), 19 (41%) were diagnosed as follicular lesion of undetermined significance (FLUS), 6 (13%) were diagnosed as follicular neoplasm (FN), 8 (17%) were diagnosed as suspicious (S), and 7 (15%) were diagnosed as papillary thyroid carcinoma (PTC). In the non-NIFT group, none was diagnosed as B, 5 (26%) cases were diagnosed as FLUS, 3 (16%) cases were diagnosed as FN, 8 (42%) cases were diagnosed as suspicious, and 3 (16%) cases were diagnosed as PTC.

FNA Diagnosis	NIFT	Invasive FVPTC (Non-NIFT)
Benign	6 (13%)	0
FLUS	19 (41%)	5 (26%)
Follicular Neoplasm	6 (13%)	3 (16%)
Suspicious	8 (17%)	8 (42%)
Papillary Thyroid Carcinoma	7(15%)	3 (16%)
Total	46	19
Male	11	4
Female	35	15
Age	29-82 (mean 51)	25-69 (mean 47)
Size	0.1-5.1 cm (mean 1.12 cm)	0.6-7.2 cm (mean 2.2 cm)
Tumor ≤1 cm	28	5

Conclusions: The majority (54%) of the FNA diagnosis for NIFT were either benign (13%) or FLUS (41%) while the majority (74%) of FNA diagnosis of the invasive FVPTC demonstrating more significant atypia (FN 16%, S 42% and PTC 16%). None of the FNA of the invasive FVPTC was benign. FNA is an effective method to identify invasive FVPTC. NIFT is a heterogenous group of lesions with the variable atypia and the FNA diagnoses may range from benign to PTC.

405 Diagnostic Utility of UroVysion for Urothelial Carcinoma of the Upper Urinary Tract

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Background: Diagnosis of urothelial carcinoma of the upper urinary tract (UCUUT) is critical. However, only few tools are available for accurate and safe diagnosis. This study aimed to determine the usefulness of UroVysion in the examination of urine cytology specimens.
Design: We prospectively enrolled 44 consecutive patients who were referred at the authors' hospitals with suspected UCUUT between 2014 and 2015. As controls, 20 patients without UCUUT were also enrolled. Selected washed or voided urine samples from the patients and controls were evaluated using a liquid-based cytological test (ThinPrep) and UroVysion. The analyses were performed using the Metafer-

Metacyte slide scanning system. At least 25 cells were analyzed for each case. Results from UroVysion were considered positive if (i) ≥ 10% cells had polysomy of three chromosomes; (ii) ≥ 10% cells had polysomy of two chromosomes and deletion of 9p21; or (iii) ≥ 30% cells had deletion of 9p21. All the samples were also evaluated by using the Paris system (TPS). The patients' characteristic and FISH data were analyzed by using the Fisher exact test.

Results: The results are shown in Tables 1 and Table 2.

Table 1: Case characteristics FISH and pathological data

	Unsatisfactory	Negative	Positive	Total
High grade UC	4	10	24	38
Low grade UC	0	3	0	3
RCC	1	0	0	1
IgG4 related	0	0	1	1
Hematoma	0	1	0	1
Negative control	0	17	3	20

UC: Urothelial carcinoma, RCC: Renal cell carcinoma

Table 2: Comparison between UroVysion and cytology by TPS

	UroVysion	Cytology (TPS)	p value
Sensitivity	63.2% (24/38)	39.5% (15/38)	.039
Specificity	82.6% (19/23)	100% (23/23)	.019
Positive predictive value	85.7% (24/28)	100% (15/15)	.124
Negative predictive value	57.6% (19/33)	50.0% (23/46)	.506

* Fisher's test ; Significant, p<.05

9 cases diagnosed as atypical cell and 4 cases diagnosed as suspicious for high grade UC by TPS were diagnosed as positive by UroVysion, respectively. A IgG4-related disease was diagnosed as positive by UroVysion, which was diagnosed as suspicious for high grade UC by TPS. Three normal control cases was diagnosed as positive by UroVysion, which was diagnosed as negative for high grade UC by TPS.

Conclusions: The results indicate that UroVysion can be a useful ancillary method to diagnose UCUUT.

406 PSMA Is a Sensitive Marker for the Diagnosis of Metastatic Prostatic Carcinoma in Cytology Specimens

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Background: Diagnosis of metastatic prostate carcinoma (MPC) in cytology specimens can be challenging due to loss of expression of commonly used markers such as prostate specific antigen (PSA) and prostate specific alkaline phosphatase (PSAP). Prostate specific membrane antigen (PSMA) is a relatively newer marker to detect prostatic carcinoma. Different from PSA and PSAP, its expression is sustained or up-regulated along tumor progression such as distant metastasis. In this study, we evaluated the expression of PSMA in cytology specimens by immunohistochemistry (IHC) in patients with a history of prostate carcinoma. The performance of PSMA in diagnosing metastatic prostate carcinoma was evaluated and compared with those of PSA and PSAP.

Design: Cytology specimens in patients with a history of prostate carcinoma were identified in our Copath database from 2005 to 2015. Slides and diagnoses of these cases were reviewed. IHC using PSMA antibody was performed in the cases with available cell blocks.

Results: 56 cases were identified in the database including 47 cases of fine needle aspiration specimens (Bone 15, lymph nodes 11, liver 8, lung 7, others 6) and 9 cases of effusion specimens. Among these, 13 cases were diagnosed as metastatic prostate carcinoma, 15 cases were diagnosed as metastatic tumor from other origins (such as lung, kidney, bladder, GI, etc), 22 cases as malignant cells present of unspecified origin, 4 cases as rare atypical cells and 2 cases as no malignant cells identified.

PSMA IHC was performed on 13 cases with previously diagnosed MPC, 24 cases with either malignant cells present of unspecified origins or atypical cells. Seven of the 24 cases were re-classified as metastatic prostate carcinoma based on positive PSMA stain. Eleven of the 13 original MPC cases were positive for PSMA. In all the 20 identified MPC cases, 18 cases were positive for PSMA (90%), 10 cases are positive for PSA (50%) and 9 cases positive for PSAP (45%). PSMA was negative in all 7 cases of metastatic carcinoma of other origins evaluated in this study (specificity 100%). In addition, PSMA has a strong membranous staining and a diffuse pattern whereas PSA and PSAP frequently show focal weak cytoplasmic stain.

Conclusions: PSMA is a highly sensitive (90%) and specific (100%) marker with a unique membranous staining pattern. PSMA is useful in diagnosing MPC in cytology specimens and provide greater diagnostic performance compared to traditional markers such as PSA and PSAP.

407 Grading of Pancreatic Neuroendocrine Neoplasms by Ki-67 Staining on Cell Blocks: Manual Count and Digital Image Analysis

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Background: Currently the WHO categorizes Pancreatic Neuroendocrine Neoplasms (PanNENs) grade according to Ki-67 index and mitotic count based on surgical pathology (SP). Controversy remains as to whether EUS-FNA cell blocks (CB) can be reliably used to grade PanNENs.

Design: A retrospective search (15 years) identified cases with both EUS-FNA and correlating SP available. 58 CBs containing >100 tumor cells were identified. Ki-67 index on CB was counted using both manual count (MC) and digital image analysis (DIA, ImmunoRatio software). The WHO grade determined on CB was compared with that on corresponding SP report. Total tumor cell number (TCN; cut offs: 500 and 2000) was used as a stratification factor.

Results: Overall, the grading concordance of CB MC and SP was 40/58(69%), and that of CB DIA and SP was 32/58(55%). WHO G1 tumors had the highest concordance: 23/31(74%) for CB MC and SP; 21/31(68%) for CB DIA and SP. Concordance for G2 tumors was: 14/23(61%) for CB MC and SP [39 undergraded and 0% overgraded]; 9/23(39%) for CB DIA and SP [52% undergraded and 9% overgraded]. G3 tumors (n=4 only) showed a concordance of 75% by CB MC and 50% by CB DIA. Among G1 and G2 tumors, the concordance improved as TCN increased, specifically, with CBs containing >2000 cells.

SP Grade	# of Cases	CB MC n(%)			CB DIA n(%)		
		G1	G2	G3	G1	G2	G3
G1	n=31	**23(74)**	7(23)	1(3)	**21(68)**	9(29)	1(3)
	TCN 100-500:n=0	-	-	-	-	-	-
	TCN 500-2000:n=14	**10(71)**	4(29)	0	**10(71)**	4(29)	0
	TCN >2000:n=17	**13(77)**	3(17)	1(6)	**11(65)**	5(30)	1(5)
G2	n=23	9(39)	**14(61)**	0	12(52)	**9(39)**	2(9)
	TCN 100-500:n=3	2(67)	**1(33)**	0	2(67)	**0**	1(33)
	TCN 500-2000:n=8	3(38)	**5(63)**	0	4(50)	**3(38)**	1(13)
	TCN >2000:n=12	4(33)	**8(67)**	0	6(50)	**6(50)**	0
G3	n=4	0	1(25)	**3(75)**	0	2(50)	**2(50)**
	TCN 100-500:n=1	0	0	**1(100)**	0	1(100)	**0**
	TCN 500-2000:n=0	-	-	-	-	-	-
	TCN >2000:n=3	0	1(33)	**2(66)**	0	1(33)	**2(67)**

Conclusions: While Ki-67 index on CB can be performed to grade PanNENs, limitation exists particularly for grade 2 tumors. CB MC had better concordance than CB DIA when compared to SP. TCN is crucial for accurate grading. Major reasons for discrepancy include insufficient sampling and background non-tumor cell contamination.

408 The Diagnostic Utility of CK7, MASPIN and MUC5AC In Distinguishing Gastric-Foveolar Type Mucinous Neoplasm of the Pancreas from Gastric Contaminants on Fine Needle Aspiration Specimens

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Background: In the evaluation of fine needle aspiration (FNA) specimens, distinguishing gastric contaminants from gastric-foveolar type mucinous neoplasm (MN) of pancreas is challenging, especially for cases without high-grade (HG) dysplasia or adenocarcinoma (ADC). On our previous study on surgical specimens, we observed 1) diffuse maspin expression in majority of pancreatic ADC and pancreatic MN with HG dysplasia; 2) normal gastric mucosa showed rare focal CK7 and diffuse MUC5AC expression. To validate the diagnostic utility of these immunohistochemical (IHC) markers on FNA specimens, the current study which includes 12 paired cytology and surgical specimens of pancreatic MN and 24 cases of normal gastric mucosa, was undertaken.

Design: Twelve paired cases of MN and 24 cases of NGM were retrieved from the pathology database, including 3 pairs of MN without HG dysplasia (group 1) and 9 pairs of MN with HG dysplasia or ADC (group 2). The FNA cases contained adequate cell block (CB) material. The IHC was performed on the paired cases (CB and surgical specimens) and normal gastric mucosa. The staining distribution was graded as negative (< 5%), 1+ (5-25%), 2+ (25- 50%), 3+ (50-75%) and 4+ (>75%). The staining intensity was noted as strong (S) or weak (W).

Results: The staining pattern for group 1 is summarized in table 1.

PAIR OF CASES	CK7 CYTO/SURG	MASPIN CYTO/ SURG	MUC5AC CYTO/ SURG
#1	2+S /2+W	0/0	4+S/4+S
#2	4+S/4+S	0/1+W	1+W/1+S
#3	4+S/4+S	1+W/1+W	4+S/4+S

The staining pattern for group 2 is summarized in table 2.

PAIR OF CASES	CK7 CYTO/SURG	MASPIN CYTO/ SURG	MUC5AC CYTO/ SURG
#1	4+S/4+S	4+S/4 S	4+S/4+S
#2	3+S/4+S	4+S/4+S	4+S/3+S
#3	4+S/4+S	4+S/4+S	4+S/4+S
#4	4+S/4+S	4+S/4+S	4+S/4+S
#5	3+S/4+S	3+S/4+S	3+S/4+S
#6	4+S/4+S	3+W/3+S	4+S/4+S
#7	3+S/4+S	3+S/4+W	2+S/2+W
#8	1+W/3+W	2+W/3+S	4+S/3+W
#9	1+W/3+W	4+S/ 4+S	4+S/4+S

The normal gastric mucosa shows weak focal staining for CK7, diffuse staining for MUC5AC, and diffuse weak staining of the superficial mucosa for maspin.

Conclusions: Our data suggests: 1) CK7+/Maspin +/ MUC5AC+or- indicates mucinous neoplasm with HG dysplasia or worse; 2) CK7+/ Maspin -/MUC5AC+or- indicates

mucinous neoplasm without HG dysplasia; 3) CK7 -/Maspin W+or- / MUC5AC + indicates gastric contaminants. Studies on a large series of cases are needed to validate these findings.

409 Relative Value of a Cytopathologist

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Background: To determine the total relative value units (RVUs), gross billing, and actual reimbursement generated by a single cytopathologist evaluating non-gynecologic cytology specimens, fine needle aspirations and consult cytology cases for the period of 1 year.

Design: The total of all CPT (Current Procedural Terminology) codes generated in anatomic pathology and recorded in Cerner CoPath was queried to identify the actual work measured in RVUs performed by a single cytopathologist (1.0 FTE, full time equivalent). The CPT codes specific to cytopathology were placed in Excel and the professional components were tallied for each of the fiscal years 2013 and 2014. Current RVUs assigned to each CPT code were used to generate a sum of RVU's for each year and then averaged. The CPT codes were selected to reflect the parts reviewed and reports generated by the responsible cytopathologist. Only those parts and accessory studies ordered and reviewed by the responsible cytopathologist were tallied. Any studies ordered on a part, but reviewed by another pathologist (flow cytometry, FISH, molecular studies) were excluded from the CPT list. Procedure codes and immediate evaluations were excluded. The summed RVUs were prorated based on the total of days the pathologist worked (4 weeks of vacation, 2 weeks of CME, 7 holidays). Finally the gross charges for the RVUs, and the reimbursed charges based on insurance payer mix were calculated.

Results: See Table 1.

CODES	RVU	GROSS CHARGE	RVU SUM ADJUSTED FOR ACTUAL WORK TIME (0.8423)	GROSS CHARGE FACTORED FOR PAYER MIX AND ACTUAL WORK TIME (PAYER FACTOR 0.31)
CYTOLOGY EXFOLIATIVE				
88104	0.56	$108	325	$19,416
88108	0.44	$116	1289	$105,330
88161	0.5	$115	181	$12,912
88312	0.54	$134	28	$2,169
88313	0.24	$85	3	$333
88305	0.75	$233	1050	$101,145
88313	0.24	$85	14	$1,520
88342	0.7	$170	901	$67,805
88346	0.86	$151	2	$118
FINE NEEDLE ASPIRATION				
88108	0.44	$108	0	$28
88173	1.39	$286	3622	$231,055
88305	0.75	$233	1904	$183,339
88342	0.7	$170	2557	$192,472
88346	0.86	$151	12	$670
CYTOLOGY CONSULT				
88312	0.54	$134	0	$35
88313	0.24	$85	2	$166
88321	1.63	$225	89	$3,819
88305	0.75	$233	30	$2,859
88342	0.7	$170	59	$4,461
Total		Annual Gross Billing of $2,936,252	12,068	$910,238

Conclusions: Our data indicated that a cytopathologist (1.0 FTE) generated 12068 RVUs in a year of work. This resulted in a gross billing of $2,936,252 with a net collection of $910,238.

410 Indefinite to Positive and Indefinite to Negative Ratios Represent Potential Quality Measures for Biliary Cytology

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Background: The atypical squamous cells (ASC) to squamous intraepithelial lesion (SIL) ratio is a common quality measure in gynecologic cytology, used to compare individual pathologists and laboratories. Similar indicators are not in common use for non-gynecologic cytology. Recently, a similar measure was proposed for thyroid cytology: the atypia (AUS) to malignant ratio. We evaluate whether any similar measures could be used as quality indicators for biliary brushing cytology.

Design: A retrospective review identified 690 biliary brushing cytology cases at our tertiary care center over 3 years. Each was categorized as negative, atypical, suspicious, or positive for malignancy. Follow-up data was collected for cases with indefinite (atypical and suspicious) diagnoses. Rates for each category and ratios of indefinite to positive (I/P) and indefinite to negative (I/N) were calculated for each pathologist and the laboratory as a whole.

Results: The cases were approximately evenly distributed among four cytopathologists. The overall diagnosis rates were: 37.9% negative (range: 35.9%-44.8%);14.6% atypical (range: 11.8%-21.0%); 5.1% suspicious (range: 3.1%-6.7%); 42.5% positive (range: 35.0%-50.4%). Definitive clinical follow-up was available in 46% of indefinite cases; of those, 75% were malignant. The I/P ratio was 0.48 (range 0.32 to 0.66) and the I/N ratio 0.53 (range 0.38 to 0.71). Individual data are shown in table 1.

		Pathologist			
	Laboratory average	1	2	3	4
Negative (%)	37.9	35.9	31.6	39.3	44.8
Atypical (%)	14.6	21.0	12.0	11.8	13.5
Suspicious (%)	5.1	4.4	6.0	3.1	6.7
Positive (%)	42.5	38.7	50.4	45.9	35.0
I/N ratio	0.53	0.71	0.57	0.38	0.45
I/P ratio	0.48	0.66	0.36	0.32	0.58
Malignant F/U (%)	73.8	80.0	71.4	62.5	81.3

Conclusions: There was substantial variation in diagnostic rates between pathologists. While the indefinite rate did not correlate with outcome, higher I/P ratios correlated with higher positive follow-up rates. The combination of indefinite rate, I/P ratio, and I/N ratio would allow laboratories to determine diagnostic precision as well as the direction of uncertainty: undercalling malignancy would increase the I/P ratio, whereas overcalling benign changes would increase the I/N ratio. These measures could be useful for inter- and intralaboratory comparisons of diagnostic precision in biliary cytology, particularly if national benchmarks were available against which to compare the data.

411 Fluorescent In-Situ Hybridization with Bile Duct Brushing Specimens: Correlation and Outcomes with Reflex Testing Algorithm

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Background: Biliary tract strictures are diagnostically challenging. Brushing can collect few cells and this quantitative limitation can make the morphologically diagnostic categorization of a bile duct brush difficult. Patients who have a negative or indeterminate result by cytology can potentially benefit from fluorescent in-situ hybridization (FISH) molecular testing. FISH testing can identify a small number of cells with molecular abnormalities restricted to malignancy. A series of patients with negative and indeterminate cytology bile duct brushing samples had reflex FISH testing and the outcomes correlated.

Design: Patients who had a biliary tract stricture and underwent bile duct brushing were identified by pathology database search for four consecutive years. Reports were reviewed and data collected. Two separate brush samples were collected in CytoLyt fixative, where the first was processed for routine cytology testing. The second vial was utilized for FISH testing, if the cytology specimen was classified as negative or indeterminate for malignancy. FISH testing utilized the UroVysion probe set (Abbott Molecular, Inc. Des Plaines, IL). The patient cohort was limited to the reflex FISH testing of negative and indeterminate cases which excluded those classified as carcinoma.

Results: 87 cases were identified. The cytology diagnostic categories included negative (72%), atypical (21%), suspicious (5%) and unsatisfactory (2%). For the benign cases, the FISH result was negative (88.9%), abnormal (6.3%) and equivocal (4.8%). Abnormal cases were those with evidence of polysomy in five or more cells. Equivocal results were those with trisomy. For atypical cases, the FISH result was negative (66.7%), abnormal (27.8%) and unsatisfactory (5.6%). For combined indeterminate categories (atypical+suspicious), the FISH result was negative (63.6%), abnormal (27.3%), equivocal (4.5%) and unsatisfactory (4.5%).

Conclusions: Reflex FISH testing algorithm for negative bile duct brush cytology categorization showed the majority (88.9%) are FISH negative. In a small group of negative cases, FISH can identify abnormal molecular findings (11.1%). For indeterminate categories (atypical+suspicious), a significant proportion demonstrated abnormal molecular findings (31.8%). Reflex FISH testing in bile duct brushing supported a negative diagnosis and identified select negative cases with molecular abnormalities, as well as finding a significant proportion of molecular abnormalities in indeterminate cases.

412 Increasing Detection of Non-Squamous Malignancies in Pap Smears in a Medical Center in Taiwan

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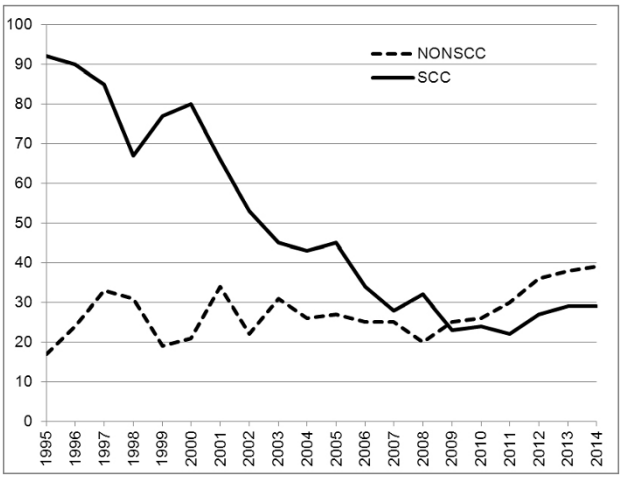
Background: Routine Papanicolaou (Pap) smear screening has made a great success in reducing the incidence and mortality of cervical cancers, especially squamous cell carcinomas. Unfortunately, the incidence of endocervical adenocarcinomas, endometrial cancers, and ovarian cancers has paradoxically risen in these years. The purpose of this study was to evaluate the Pap smears in detecting cancers other than cervical squamous cell carcinomas.

Design: In Taiwan, the nationwide annual Pap smear screening program for women aged 30 years and older has been launched since 1995. All malignant results of Pap smears from 1995 to 2014 were retrieved from the computerized database of a medical center in Taiwan. The clinical information as well as the corresponding pathological results was obtained from the medical records.

Results: There were 515593 Pap smears during the period of 20 years. Among them, a total of 988 squamous cell carcinomas and 532 non-squamous cell malignancies were found. Detection of non-squamous cell malignancies exceeded squamous cell carcinomas since 2009.



The most common origins of the non-squamous malignancies were extra-uterine (29%), endometrial (25%), and endocervical adenocarcinomas (22%). Approximately 80% of the extra-uterine cancers were originated from the pelvic organs, e.g. ovary (49%), colon-rectum (19%), and urinary bladder (10%). The most common distant metastases were from the breast (7%) and lung (5%).

Primary origins of extra-uterine cancers	N (%)
Ovary	76 (49)
Colorectum	30 (19)
Urinary bladder	16 (10)
Breast	11 (7)
Lung	7 (5)
Stomach	4 (3)
Fallopian tube	4 (3)
Unknown primary	3 (2)
Pancreas	2 (1)
Lymphoma	1 (1)
Total	154 (100)

Conclusions: Due to a marked reduction of the incidence of cervical squamous cell carcinomas, non-squamous malignancies have become the majority of malignant Pap smears in our practice. Moreover, secondary involvement of breast or lung cancers in the cervix is not uncommon. Therefore, to recognize non-squamous malignancies in Pap smears has become an important issue.

413 Diagnostic Accuracy of Pancreatic Fine Needle Aspiration and Evaluation of Discordant Cases

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Background: False-positive pancreatic fine needle aspiration (FNA) diagnoses may result in overtreatment of patients, including complicated surgical procedures with high morbidity and mortality rates. Few data exist on discordant diagnosis rates and causes of error, yet this data is important in order to improve diagnostic accuracy.

Design: 721 pancreatic FNA cases performed and/or reviewed at our institution over 3 years were identified. Of these cases, 213 had a confirmatory resection specimen, on which cytology-histopathology correlation was performed. The discordant diagnosis rates were determined.

Results: The majority of the 213 cases with a confirmatory resection specimen were initially categorized on cytological examination as positive for neoplasm (n=141, 66%), with the remaining categorized as suspicious (n=13, 6%), atypical (n=23, 11%), benign (n=28, 13%), and insufficient (n=8, 4%). Analysis of the positive and suspicious cases showed 4 discordant cases, resulting in a discordant diagnosis rate of 2.6%. No false negative cases were identified. The discordant cases involved 1 FNA diagnosis of suspicious for neuroendocrine tumor with the resection specimen showing serous microcystic adenoma, 1 FNA diagnosis of suspicious for mucinous neoplasm with the resection specimen showing metastatic carcinoma consistent with breast primary, and 2 FNA diagnoses of adenocarcinoma with the resection specimens showing an intraductal tubulopapillary neoplasm and a neuroendocrine tumor with PanIN2. In 2 of the 4 cases, the FNA material was reviewed by 2 cytopathologists, and the presence of neuroendocrine cells and malignant epithelium were confirmed. However, in retrospect the findings likely represented islet cell sampling and foci of PanIN adjacent to the targeted pancreatic lesion. In the case of metastatic breast carcinoma, no history was provided.

Conclusions: In spite of the high accuracy of FNA cytology diagnosis of pancreatic lesions, avoidance of certain pitfalls may reduce the likelihood of making a false positive diagnosis. Aspiration of adjacent non-lesional tissues should be carefully considered, especially in limited samplings. Correlation with radiographic imaging and clinical history may also assist in avoidance of these errors.

414 Utility and Pitfalls of GATA3 Immunocytochemistry for Diagnosis of Breast and Urothelial Carcinomas in Cytology Specimens

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Background: Cytologic diagnosis of metastatic carcinoma of breast or urothelial origin is not always straightforward. Although studies have shown that GATA3 is highly and specifically expressed in primary and/or metastatic breast and urothelial carcinomas in surgical specimens, only a few studies address the utility of GATA3 in cytologic diagnosis. The impact of GATA3 expression in various cytologic sample types (effusion fluid vs. FNA and cell block vs. smear) has also been understudied.

Design: We retrospectively searched our pathology database for cytologic cases that used GATA3 immunostaining during diagnostic workup between 2013 and 2015. We examined GATA 3 expression in carcinomas of various origins, and its correlation with ER and PR in breast carcinomas.

Results: A total of 178 cases (143 FNA and 35 fluid samples) were identified, including 87 metastatic breast carcinomas, 22 metastatic urothelial carcinomas, and 69 malignant neoplasms of other origins. Of the 87 metastatic breast carcinomas, 64 cases (74%) were positive for GATA3 staining, 22 (25%) were negative, and 1 (1%) showed equivocal staining. ER was positive in 55 cases (55/86, 64%), PR was positive in 37 cases (37/82, 45%), and 61 cases (61/85, 72%) were positive for ER and/or PR. Breast carcinomas with positive GATA3 stain had higher positive rates for ER and PR than did those with negative GATA3 stain (75% vs 27% for ER, 52% vs 14% for PR, respectively). Of the 20 triple-negative breast carcinomas, 9 (45%) were GATA3-positive. GATA3 was performed on cell blocks in 67 cases and on direct smears in 20 cases, with positive rates being 70% (47/67) and 85% (17/20), respectively. Staining was positive in 72% of FNA samples (41/57) and in 77% of fluid samples (23/30).

Of the 22 metastatic urothelial carcinomas, all cases were positive for GATA3 staining (21 cases using cell blocks and 1 using smear slide).

Of the 69 malignancies of other origins, GATA3 staining was found in 6 cases (9%), including ovarian carcinoma (1), squamous carcinoma (1), skin adnexal carcinoma (1), thymic carcinoma (1), and carcinomas of unknown origin (2).

Conclusions: GATA3 is useful to detect carcinomas of breast and urothelial origins in cytologic specimens with high specificity. Its expression in carcinoma of breast primary is higher than that of ER and comparable to that of ER and/or PR. GATA3 is also helpful to detect metastatic triple-negative breast cancers. Both cell block and smear preparations can be reliably used for GATA3 staining.

415 The Bethesda System Class III Thyroid Nodules: Follow Up Data and Conclusions Based on 13,194 Thyroid Fine Needle Aspirates

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Background: Fine needle aspiration (FNA) is considered to be the best screening test for thyroid nodules. The Bethesda System Thyroid diagnostic categories have standardized classification of nodules. The third (III) category of atypia of undetermined significance and follicular lesion of unknown significance (AUS/FLUS) is used to triage cases which do not meet malignant criteria but cannot be diagnosed as benign either. The goal of this study was to assess follow up, and evaluate our diagnostic criteria for category III based on a large series of cases.

Design: We conducted a search through our institutional database from 01/01/2008 to 04/30/2015 that included the words "atypia of undetermined significance" and "atypical follicular cells present". "Suspicious for follicular neoplasm" cases were excluded.

Results: Our findings are represented in Table 1. Our search yielded 206 (1.6%) AUS/FLUS cases from 13,194 thyroid FNAs from 2008-2015. Of these 206 cases, 158 had follow-up data with a total of 203 diagnoses. Molecular studies were not performed in any of the cases.

Table 1. Follow-up results	**Overall (%)**	**FNA (%)**	**Surgery (%)**
Total	203 (100)	41 (100)	162 (100)
Benign thyroid nodule	70 (34)	23 (56)	47 (29)
PTC	61 (30)	3 (7)	58 (36)
Classic	27 (13)	3 (7)	24 (15)
Follicular variant	16 (8)	0 (0)	16 (10)
Microcarcinoma	16 (8)	0 (0)	16 (10)
Oncocytic variant	2 (1)	0 (0)	2 (1)
Follicular carcinoma	22 (11)	3 (7)	19 (12)
Classic	11 (5)	1 (2)	10 (6)
HÃ¼rthle-cell type	11 (5)	2 (5)	9 (6)
Follicular adenoma	16 (8)	0 (0)	16 (10)
Hashimoto's thyroiditis	13 (6)	1 (2)	12 (7)
HÃ¼rthle cell adenoma	8 (4)	0 (0)	8 (5)
Granulomatous thyroiditis	1 (0.5)	0 (0)	1 (0.5)
Graves disease	1 (0.5)	0 (0)	1 (0.5)
Atypia of unknown significance	4 (2)	4 (10)	0 (0)
Unsatisfactory / Scant	7 (3)	7 (17)	0 (0)
Follow up average (months)	7.74	13.04*	2.44*
*p<0.001			

Conclusions: While the rate of atypia of unknown significance (AUS/FLUS) in our series remains relatively low (1.6%), 41% of these cases are diagnosed as malignant on follow-up, and 8% were follicular adenomas. Applying strict diagnostic criteria for category III helps us streamline cases and avoid unnecessary surgical procedures.

416 Performance of the Afirma Gene Expression Classifier in the Evaluation of Cytologically Indeterminate Thyroid Nodules: An Institutional Experience

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Background: Molecular testing of thyroid fine-needle aspiration (FNA) specimens has the potential to improve diagnostic yield for the 15-30% of cases with indeterminate cytology. The Afirma Gene Expression Classifier (GEC) reports a negative predictive value (NPV) of 94-95% and a positive predictive value (PPV) 37-38% for indeterminate nodules, identifying aspirates with the greatest risk of malignancy while sparing most patients from unnecessary surgery. This study reviews the authors' institutional experience with Afirma in an academic medical center.

Design: A cohort of 939 thyroid FNAs performed from 2013 to 2015 was selected from the study files and relevant information was recorded and analyzed. 240 of these aspirates were diagnosed as atypia of undetermined significance/follicular lesion of undetermined significance (Bethesda category III). Of these, 69 indeterminate aspirates had material collected for GEC analysis. Follow-up was either clinical or surgical.

Results: Of the 69 specimens, 2 (2.9%) contained insufficient mRNA, leaving 67 Afirma results for analysis. Of these, 24 (35.8%) were benign and 43 (64.2%) were suspicious. 19 patients diagnosed with GEC-suspicious nodules underwent surgery; malignancy was confirmed in 8 (42.1%) of these cases and 11 (57.9%) cases were benign. All GEC-benign nodules (n=24) and only those GEC-suspicious nodules that underwent surgery (n=19) were included for analysis. Based on clinical follow-up of benign cases for a median of 5.8 months with no false-negative results: sensitivity=100%; specificity= 68.6%; prevalence of malignancy=18.6%; NPV of benign GEC= 100%; PPV of suspicious GEC= 42.1%, accuracy=74.4%.

Conclusions: In this study, the Afirma GEC demonstrates a lower than expected malignancy rate within GEC-suspicious nodules (institutional prevalence of malignancy for Bethesda category III FNAs=37%). While Afirma is useful to rule out malignancy, a high rate of unnecessary surgery was evident. For GEC-suspicious nodules, further confirmatory tests should be performed prior to definitive surgery.

417 Ultrasound Guided Fine Needle Aspiration of Salivary Gland Nodules by Cytopathologists at The University of Toledo Medical Center. A Fast, Safe, and Accurate Approach

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Background: Ultrasound guided fine needle aspiration (US-FNA) is the standard method for sampling salivary gland nodules (SGN). Increasingly, this procedure is being performed by cytopathologist. Our study evaluates the accuracy, safety, and results of US-FNA performed by cytopathologists at our institution over a 58-month period.

Design: US-FNAs of all SGN performed by cytopathologists between October 2010 and August 2015 were retrospectively reviewed. Clinical data, number of passes, complications, adequacy, diagnosis, tissue correlation, and turnaround times were analyzed. The aspirated material was obtained using 25 G x 1½ needles. Smears were stained for immediate evaluation with Diff-Quik. Additional smears were fixed in alcohol for Papanicolaou stain. Reports included final diagnosis, clinical data, ultrasound findings, procedure notes, and a microscopic description.

Results: A total of 63 salivary gland US-FNAs from 56 patients were evaluated. The size of the nodules ranged from 0.6 to 7.0 cm with an average size of 2.4 cm. Nodules were sampled an average of 3 times with the number of passes ranging from 1 to 6. No complications were reported. All samples (100%) were adequate for evaluation. Of the 63 cases, 21 (33.3%) were non-neoplastic, 33 (52.4%) were benign neoplastic, and 9 (14.3%) were malignant. None of the non-neoplastic cases had tissue correlation. There were 17 (51.5%) cytologically benign neoplastic cases with tissue correlation, all which were histologically confirmed as such including 11 pleomorphic adenomas (64.7%), 4 Warthin tumors (23.5%), 1 lipoma (5.9%), and 1 basal cell adenoma (5.9%). Of the 9 malignant cases, 6 (66.7%) had tissue correlation and 3 (33.3%) had corresponding flow cytometry. In all 9 cases (100%), the cytologic diagnosis was confirmed. This included 2 cases of metastatic carcinoma, 1 case of acinic cell carcinoma, 1 case of mucoepidermoid carcinoma, 1 case of mammary analog secretory carcinoma, 1 case of poorly differentiated carcinoma, and 3 cases of non-Hodgkin lymphoma. A final diagnosis was reported in 82.5% of the cases within 2 business days with the majority (71.4%) of those cases being reported in one business day or less.

Conclusions: US-FNA performed by cytopathologists was proved to be a safe, accurate, fast, and effective diagnostic modality at our institution.

418 Genetic Alterations Detected in Urine Cytology Specimens from Patients with High Risk Non-Muscle Invasive Urothelial Carcinoma Treated with BCG

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Background: High Risk (HR) non-muscle invasive urothelial carcinoma (NMIUC), including high grade papillary carcinoma, carcinoma in-situ and superficially invasive urothelial carcinoma, are typically treated with the instillation of intravesical BCG. This therapeutic management can lead to the cure of disease or allow local control of the disease. However, approximately 20% of patients with HR NMIBC do not respond to this treatment. These patients are at higher risk for progression to muscle invasive tumors. Substratification of patients with HR NMIUC in patients represents a major need as, currently, it is not possible to predict who will respond favorably to BCG and who might benefit from early aggressive treatment, such as cystectomy, to achieve cure. We propose to study urine cytology specimens from patients with HR NMIUC and analyze a large panel of genes with the intent to identify patients who might not benefit from BCG treatment.

Design: Fifty-three slides from forty-two patients diagnosed with HR NMIUC and treated with intravesical BCG were selected for this study. All specimens were contained at least 1,000 cells neoplastic each and tumor cells represented over 50% of the specimen cellularity. Histologic confirmation was available in all cases. The patients were subdivided into 3 categories: responders, with relapsed disease and refractory to treatment. Clinical follow up was available for all patients.

DNA was extracted from the cytology slides and subjected to next generation sequencing (NGS) analysis using a customized targeted exome capture assay composed of 341 genetic abnormalities, including oncogenes, tumor suppressor genes, and components of pathways deemed actionable by targeted therapies. Captured pools were sequenced on an Illumina HiSeq 2000.

Results: The patient population included 8 responders, 7 with relapsed disease and 22 with refractory disease. The average amount of DNA obtained from each slide ranged from 23 to 2080ng, with an average of 359ng. The number of genetic alterations ranged from 2 to 87 with an average of 18.4. Specimens from responders contained no ARID1A mutation or MDM2 amplification in any case. Mutations in AKT2, ARID1A and EPHA3 were significantly more frequent ($p<0.05$) in patients refractory to treatment in comparison to responders.

Conclusions: Urine specimens are suitable for NGS analysis. There are genetic alterations significantly more common in patients with HR NMIUC refractory to BCG while certain mutations are absent in patients who responded to BCG treatment in our series.

419 Cytomorphology of Clear Cell Papillary Renal Cell Carcinoma

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Background: Clear cell papillary renal cell carcinoma (CCPRCC) is a recently recognized subtype of RCC, although it shares some morphologic and immunohistochemical markers with clear cell RCC and papillary RCC. Its cytomorphology of fine needle aspiration (FNA) or touch preparation (TP) of needle core biopsy (NCB) has not been well studied and published.

Design: Renal FNA/NCB cases in Northwestern Memorial Hospital were reviewed and 6 CCPRCC cases were identified. The FNA/TP cytomorphology of each case was studied, which includes cell arrangement patterns, nuclear characteristics, cytoplasmic features, and background.

Results: The FNA/TP smears showed that the tumor cells were arranged in small nests (100%), 3-dimensional clusters (83%), papillary (50%), tubule/acini (50%), large sheets (17%) and singly (67%). The tumor cells were columnar in shape (100%) and possibly polygonal (67%) with small round or oval nuclei located at one pole of cells (100%). Nuclei contained evenly distributed, fine granular chromatin and showed smooth nuclear membrane (100%) (Grade 1). Rare tumor cells had conspicuous nucleoli (33%). Naked tumor cells were seen in 33% of cases. The tumor cells had moderate amount of cytoplasm containing comparatively uniform small vacuoles (100%) with ill-defined cytoplasmic borders (100%). The cytoplasm might be also clearing (50%) or delicate (83%), and rarely contained granules (33%). Rare mitoses were seen in 17% of cases. Small vacuoles (67%), macrophages (67%), and necrosis (33%) could be seen in the background. Vessels were seen in the papillary cores, transversing nests of tumors or around tumors. Immunochemical studies showed that the tumor cells were diffused positive for CK7 (100%) and CA IX (100%, cup-like membrane), and focally positive for AMACR (40%) and CD10 (50%, lumen and reverse cup-like as compared to CA IX). The tumor cells were negative for CD117.

Conclusions: FNA smears/TP of CCPRCC showed unique cytologic features, papillary or tubule/acini patterns, columnar cell shape, low grade (Grade 1) nuclei located at one pole of cells, moderate amount of cytoplasm containing comparatively uniform small vacuoles that were also present in the background. These cytologic features are helpful to diagnose CCPRCC on FNA smears. If cores or cellblocks are available, immunochemical panel including CK7, CA IX, CD10 and AMACR are helpful for the diagnosis of CCPRCC. This subtype of RCC should be differentiated from other types of RCC due to its low grade indolent behavior.

420 Prospective Tonsillar Pap Smears with High Risk HPV Testing of 41 Adults with Neck Mass and Unknown Primary

Megan G Lockyer, Matt Prall, Andrew Veyliotti, Terry K Morgan. OHSU, Portland, OR.

Background: The incidence of high risk human papillomavirus (hrHPV) related oropharyngeal carcinomas (OPC) is increasing, perhaps due to new diagnostic awareness and testing methods. In the United States, the incidence of metastatic hrHPV related OPC is expected to exceed metastatic cervical cancer by 2020. Therefore, similar to proven cervical cancer screening methods, we hypothesized that tonsillar brushings with hrHPV testing may provide predictive value in a prospective study of previously undiagnosed adults.

Design: Prospective study of patients with no history of OPC presenting to Oregon Health & Science University with a neck mass of unknown etiology for fine needle aspiration (FNA) biopsy. Tonsillar ring brushings were obtained using a commercially available Cytobrush (Cooper Surgical) that we manually curved at the end to provide wider surface area. The sample was then transferred to Preservcyt solution (Hologic) and the FNA of the neck mass was performed. Clinical outcomes were based on FNA diagnoses, surgical biopsies, and follow-up. We expected a 10% prevalence of hrHPV positive OPC in our high risk clinic. A portion of the fixative solution was used to make ThinPrep slides (Hologic) for cytologic evaluation, a second equal portion was used for hybrid capture 2 (hC2) hrHPV testing (Qiagen/Digene). The third portion was banked. Adequacy was defined as the presence of at least 5000 cells in the ThinPrep slide and the presence of *Actinomyces* organisms suggestive of tonsillar sampling.

Cytology was evaluated for adequacy and classified as negative, atypical, or carcinoma by a cytotechnologist, cytology fellow, and cytopathologist blinded to outcomes. Discrepancies were settled by consensus.
Results: 41 subjects (19 women and 22 men) met adequacy criteria. Eight cases were later diagnosed with biopsy proven hrHPV positive OPC (8/41, 19%). Carcinoma was more common in males (7/22) compared with females (1/19) (Fisher's exact p=0.05). 5/8 cases had hrHPV positive oral paps. One was indeterminate (RLU 0.97), and two paps were false negatives. False negatives were later determined to be from the base of tongue and an unknown primary. The relative risk for hrHPV OPC diagnosed by tonsillar pap was 42 [2.5-684] (p=0.01) with moderate sensitivity (71%) and good specificity (100%). Cytology alone revealed only one OPC.
Conclusions: Prior studies have brushed grossly visible OPCs, but our study is the first to screen for hrHPV positive OPCs in high risk patients with unknown primaries. Our data suggest hrHPV testing is promising, but insufficient sampling of Waldheyer's ring may to be related to less sensitivity.

421 EGFR, KRAS, and ALK: Are Really Mutually Exclusive?. Report of Five Cases Harboring Two Different "Theoretically Exclusive" Double Mutations Diagnosed by FNA Cytology

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Background: Driven by therapeutic advances, a revolution is taking place in the lung cancer field that has major implications for pathologic diagnosis and tissue management. More than 70% of the cases are unresectable at diagnosis. Thus small biopsies and FNA cytology are in the front line of mutational status studies in NSCLC patients. Although considered mutually exclusive, simultaneous presence of *ALK* and *EGFR* mutations as well as *EGFR* and *KRAS*; and *KRAS* and *ALK* have been identify in a small number of patients.
Design: To report the cases with coexistence of two molecular alterations from our series of 650 NSCLC patients diagnosed by FNA cytology with routine molecular analysis, consistent of *EGFR, KRAS, BRAF,* and *ALK*. All the molecular analysis were performed on stained smears as previously reported.
Results: Table 1 summarizes patient's characteristics. Four patients had stage IV lung adenocarcinomas, and one metastatic lung squamous cell carcinoma. diagnosed by FNA cytology from different metastatic sites. In one case *EGFR* mutations were detected and the patient had a four year response to TKIs. At relapse an additional T780M *EGFR* mutation and rearrangements of *ALK* appeared together with her initial *EGFR* mutation. The other four cases showed from the initial diagnosis coexistence of *KRAS* and *ALK*; and *EGFR* and *KRAS* mutations respectively.

Case #	Gender	Age	Smoking Habit	Diagnosis	EGFR	KRAS	ALK
1	Female	70 74	no	Adenocarcinoma Adenocarcinoma with signet ring cells	L858R L858R + T790M	WT WT	ND 26% positive cells
2	Female	80	no	Adenocarcinoma stage IV	WT	G12C	15% positive cells
3	Male	45	no	Adenocarcinoma stage IV	WT	G12D	76% positive cells
4	Male	43	yes	Squamous stage IV	R836R*	G12D	negative
5	Male	49	yes	Adenocarcinoma stage IV	Del 19	G12D	negative

Conclusions: Although infrequent, coexistence of *ALK* rearrangement and *EGFR* and/or *KRAS* mutations, as well *EGFR* and *KRAS* mutations does occur. FNA cytology plays an especial role. It is mandatory to manage the cytological samples for molecular studies so that no patient is excluded from the possibility of receiving targeted therapies.

422 The Impact of Application of the Proposed Paris System on Concordance Rates and Frequency of Diagnosis of Low-Grade Urothelial Carcinoma on Voided Urine Cytology

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Background: Voided urine cytology (VUC) is a convenient method of triaging patients with urinary symptoms and in surveilling patients with a history of urothelial carcinoma. However, the sensitivity and negative predictive value in detecting low-grade urothelial carcinoma (LGUC) is low and there is no standardized method of diagnosing LGUCs in VUCs. The Paris Consensus Conference has proposed a classification system, the Paris System, for urine cytology that includes a diagnostic category for LGUC. We sought to determine intra- and inter-observer concordance rates among cytopathologists (CPs) before and after application of the Paris system and to determine if application of its proposed diagnostic criteria for LGUC increased the frequency with which LGUCs are diagnosed in VUCs.
Design: Electronic pathology records from January 2011 to May 2013 identified 61 patients with VUCs collected within a 3 month period prior to a biopsy diagnosis of LGUC. Patients with foci of high-grade UC were excluded. These 61 VUCs and an additional 12 VUCs from patients with benign follow-up were blindly reviewed by 3 cytopathologists (CPs). Concordance was considered to represent agreement among all CPs. Slides were re-reviewed after a tutorial was given to emphasize diagnostic criteria for LGUC in the proposed Paris System, including 3D urothelial cell clusters with nuclear overlap and high numbers of monotonous non-superficial cells in VUCs.
Results: After initial review, there was an overall 70% concordance rate among the 3 CPs (83% in negative cohort, 67% in LGUC cohort). Of the concordant results, 42 were negative and 9 were atypical. There were no definitive diagnoses of UC. In the post-tutorial review, each CP changed approximately 1/3 (27%, 38%, and 39%) of their original diagnoses, with 70% of the changes representing negative/atypical diagnoses to LGUC. It is notable that based on proposed diagnostic criteria, all CPs changed the same 2 of 12 VUCs from patients with benign follow-up from negative to LGUC diagnoses. The overall concordance rate after the post-tutorial review among the 3 CPs was 64% (83% in the negative cohort, 60% in the LGUC cohort).
Conclusions: Based on our preliminary findings, application of proposed diagnostic criteria in the Paris System does not increase the concordance rate among CPs. Although the criteria increased the detection of LGUCs in VUCs, they also resulted in false positives, which may result in an increase in unnecessary procedures.

423 Pancreatic Metastases: Potential for Misdiagnosis on Fine Needle Aspiration

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Background: Metastatic tumors involving the pancreas are uncommon and may be problematic on fine needle aspiration biopsy, particularly in absence of a clinical history of tumor elsewhere.
Design: The pathology database at a single academic medical center was searched for surgical specimens with a diagnosis of metastatic neoplasm involving the pancreas. Clinical and pathologic material was reviewed to determine clinical history, level of clinical suspicion and pathologic diagnosis.
Results: We identified 34 resection cases with available material for review, 11 of which had prior fine needle aspiration (FNA) biopsy. Renal cell carcinoma accounted for the most common primary site (n=15) followed by colorectal carcinoma (n=7), ovarian carcinoma (n=4), breast carcinoma (n=2), and other (n=6). In only 5 cases was a clinical history of prior carcinoma provided, although it was known in 9 cases. FNA diagnosis was accurate in all cases with a provided clinical history of primary tumor elsewhere. In 2 cases of metastatic breast carcinoma, there was no provided clinical history and no clinical suspicion of metastasis; in both cases, an FNA diagnosis of "consistent with" or "suspicious for" primary pancreatic neoplasia was rendered. The correct diagnosis was not established until the resection specimen was examined. In one of these cases, metastatic lobular breast carcinoma exhibited signet ring-like morphology, contributing to misinterpretation as a primary pancreatic mucinous neoplasm.
Conclusions: Metastatic tumors involving the pancreas are uncommon but may be problematic on fine needle aspiration biopsy, particularly in absence of a clinical history of tumor elsewhere. This series highlights the importance of the clinical history in the evaluation of pancreatic masses in order to avoid unnecessary surgery, especially when encountering metastatic lesions sharing morphologic overlap with primary pancreatic neoplasms.

424 Suitability of Endobronchial Ultrasound Guided Transbronchial Needle Aspiration (EBUS-TBNA) Samples for Diagnosis, Staging and Molecular/NGS Testing in Metastatic Malignancies Involving the Mediastinum

Valerica Mateescu, Ritu Nayar, Nike Beaubier, Ajit Paintal. Northwestern Memorial Hospital, Chicago, IL.
Background: EBUS-TBNA is increasingly used for the diagnosis and staging of both benign disease as well as lung cancer and other malignancies. The aim of our study was to review our adequacy rate for this specimen type in the context of rapid on site evaluation (ROSE) and to assess the suitability of EBUS-TBNA specimens for both single gene molecular assays and next generation sequencing (NGS).
Design: 215 consecutive lymph node EBUS-TBNA specimens from 100 patients performed between 2/2015 and 8/2015 were retrieved from our institution's medical record. EBUS-TBNA was performed using an Olympus EBUS-TBNA 22 gauge needle by an experienced pulmonologist. In most cases, direct smears were made for ROSE with additional material processed as a cell block using the plasma thrombin technique. When indicated in cases of non-small cell lung carcinoma (NSCLC), KRAS and EGFR testing were performed via real-time PCR and if wild-type, reflexed to ALK FISH. In cases of metastatic melanoma (MM), BRAF mutation testing was performed via real-time PCR. All molecular testing was performed on cell block material.
Results: An average of 3 passes each were performed on an average of 3 lymph node stations per patient. Adequacy rates for diagnosis were superior in specimens with ROSE (169/177, 90%) than in those without (29/38, 76%). 51 patients had metastatic malignancy including lung adenocarcinoma (ACA) (19), NSCLC,NOS (6), lung squamous cell carcinoma (9), small cell carcinoma (5), MM (2), and other malignancies (10). 10 patients had granulomatous lymphadenitis.
Molecular testing was possible in specimens from 23/26 (88%) of patients where it was indicated including MM (2) and NSCLC (NSCLC,NOS and ACA) (24). An EGFR, KRAS or ALK lesion was identified in 53.5% of tested NSCLC cases. No BRAF lesions were found in tested MM cases. Following macrodissection, an average tumor cellularity of 56% (10%-90%) was present in cell blocks used for molecular testing. The average DNA yield per case was 47ng/µl (range 15-160).
Conclusions: Our study demonstrates that EBUS-TBNA consistently provides adequate material for morphologic diagnosis as well as molecular studies, particularly in conjunction with ROSE. Based on the specimen requirements listed by the two

major NGS platform manufacturers (10-50 ng/µl depending on the instrument, library preparation method and panel), specimens obtained via our methodology would be suitable for NGS based testing in many if not most cases depending on the platform.

425 Follicular Lymphoma Diagnosis by Fine Needle Aspiration Biopsies in a Cancer Center

Zulfia McCroskey, Joseph D Khoury, John M Stewart, Nancy Caraway. MD Anderson Cancer Center, Houston, TX.

Background: The diagnosis and grading of follicular lymphoma (FL) by fine needle aspiration biopsy (FNAB) is controversial. Whereas it offers superior cytomorphologic details that favor grade evaluation, this sampling modality does not permit evaluation of tissue architecture. The differential impact of the advantages and limitations of FNAB in the diagnosis of FL have not been systematically evaluated in comparison to core needle biopsy (CNB) results. In this study, we evaluate the accuracy of diagnosing and grading FL by FNAB in a cohort of patients seen at a large cancer center.

Design: We identified retrospectively cases diagnosed as FL by CNB between 1/2012 and 7/2015. Inclusion criteria included having a concurrent FNAB and adequate cell counts to perform multiparameter flow cytometric analysis (FCM). The FCM results were available for interpretation of both FNAB and CNB.

Results: The study group included 346 cases that met the selection criteria; they included 288/346 (83%) low-grade (LG), 45/346 (13%) high-grade (HG), and 13/346 (4%) not graded FLs.

		FNA					
		LBCL	BCL, NOS	FL HG	FL LG	FL	Insufficient
CNB	FL HG	9	4	14	3	13	2
	FL LG	2	7	8	191	60	20
	FL	2	0	0	4	7	0

There were 80/346 (23%) cases that were classified as FL but not graded on cytology; these cases were included in the analysis. For this study, all FLs graded as 1 or 2 were grouped into the LG category. FNABs diagnosed as grade 3 and HG FLs were grouped together in the HG FL category, and FNAB diagnosed as large B-cell lymphomas (LBCL) of follicular center cell origin were grouped separately. The sensitivity for grading FL by FNAB was 71 % for LG, 33% for HG FL, and 54% for HG category (combined HG FL and LBCL).

Conclusions: The accuracy for grading LG FLs by FNAB is high when an adequate sample for cytomorphology and FCM is obtained. On FNAB, it is more difficult to differentiate HG FLs from diffuse LBCL of germinal center origin due the cytomorphologic and immunophenotypic overlap and lack of architecture.

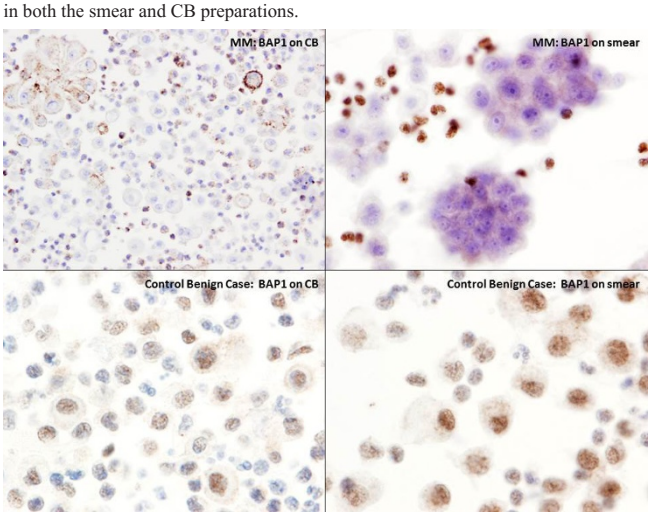
426 Utility of BRCA1-Associated Protein 1 (BAP1) Immunoperoxidase Stain in Cytology Smear/Cell Block Preparations to Differentiate Benign Versus Malignant Mesothelial Proliferations

Zulfia McCroskey, Gregg Staerkel, Sinchita Roy Chowdhuri. MD Anderson Cancer Center, Houston, TX.

Background: Loss of BAP1 staining has recently been described as a highly specific marker for distinguishing malignant mesotheliomas (MM) from benign mesothelial proliferations (BMP). The aim of this study was to evaluate the utility of BAP1 staining in effusion samples using cytologic smears and cell block (CB) preparations.

Design: We retrospectively searched the database at our Cancer Center for smear and CB preparations from cases of MM (n=21) and BMP (n=11). A Papanicolaou stained smear and CB section from each case were selected for BAP1 staining (Clone C-4, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The results were scored as nuclear BAP1 staining, absent or present. Cytoplasmic staining was considered nonspecific and positive staining in non-neoplastic cells was noted as internal control.

Results: In patients with a diagnosis of MM, 17/21 (81%) cases showed a loss of BAP1 in both the smear and CB preparations.



Of the 4 remaining cases with retained BAP1, 2 had positive nuclear stain in the MM cells, on both smears and CBs, while 2 retained BAP1 only on the smear. In the control group, 11/11 (100%) cases showed nuclear BAP1 in the CB, with 10/11 (91%) showing nuclear staining in smears, while 1 smear showed high background staining and was discarded as equivocal . Most smears (28/31; 90%) and a subset of CBs (18/31; 58%) had non-neoplastic cells with nuclear stain that served as an internal control. Sensitivity/ specificity for BAP1 loss in MM was 81%/91% and 90%/100% for smear and CB, respectively.

Conclusions: Although, loss of BAP1 can be used as a highly specific marker to distinguish benign versus malignant mesothelial cells, positive staining cannot be used to exclude a diagnosis of MM. CB preparations had higher sensitivity/specificity than smears in identifying MM using BAP1.

427 Pancreatic Endoscopic Ultrasound-Guided Fine Needle Aspiration: The Impact of Rapid On-Site Evaluation

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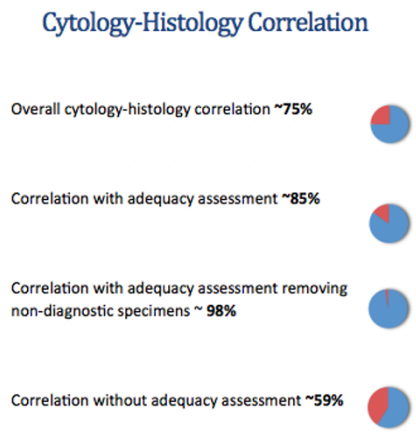
Background: Endoscopic ultrasound guided fine-needle aspiration is often used for the evaluation of patients with pancreatic lesions and rapid on-site evaluation (ROSE) is frequently utilized for adequacy assessment and specimen triage purposes. Although these are potential benefits, ROSE increases time demands placed on the Pathology staff. Considering that our institution has seen nearly a 12% annual growth rate of EUS procedures over the past five-years, we retrospectively compared cases utilizing and not utilizing ROSE.

Design: A total of 284 EUS FNA cases were seen at our institution over a 5 year period. The number of cases using and not using ROSE was analyzed comparing specimen adequacy and cytology-histology correlation rates as a means to assess diagnostic yield and interpretation performance.

Results: Of the 284 cases, 163 (57.4%) used ROSE and 153 of these were adequate. The 10 non-diagnostic cases were interpreted as such during ROSE. Of the 121 non-ROSE cases; 27 were non-diagnostic and 94 were adequate. However, 20 of these 94 adequate cases were "limited for evaluation". A total of 110 cases had follow-up material in our databases and the overall cytology-histology correlation rate was 75.5%.



Non-correlating cases were due to cytologic sampling variances. 254 cases had cell blocks prepared. Diagnoses were dependent on cell block material in only 3 cases (1.18%).

Conclusions: ROSE is advantageous as it allows for "real-time" evaluation. If the desired target is not adequately sampled, additional passes can be made. In our study, nearly 40% of cases without ROSE were either limited or non-diagnostic and cytology-histology correlations varied when taking ROSE into account (59% compared to 85% with ROSE). Fine-needle aspiration performance is dependent on adequacy and ROSE favorably impacts adequacy which decreases the need for repeat procedures. Additionally, we found only 1% of diagnoses were dependent on cell block material, highlighting the importance of obtaining diagnostic material on the cytologic smears for optimal procedure performance.

428 Risk-Stratified Triage of Salivary Gland Fine-Needle Aspiration Specimens

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Background: Classification of salivary gland tumors by fine-needle aspiration (FNA) is challenging due to the diversity and morphologic overlap of the >30 entities recognized by the WHO. Nevertheless, FNA is a useful tool for pre-operative planning, especially in the superficial parotid gland where benign (B) and low-grade (LG) malignant tumors are typically treated by limited excision, while high-grade (HG) malignant tumors are treated by total parotidectomy potentially accompanied by neck lymph node dissection and adjunctive therapies. Yet little data exist on the ability of salivary gland FNA to distinguish B/LG tumors from HG tumors.

Design: We identified all salivary gland FNAs with subsequent surgical excision at our institutions over a 10-year period (January 2005-September 2015). Cases with an atypical lymphoid proliferation or lymphoma were excluded. FNA diagnoses were classified into four categories: non-diagnostic, B/LG, HG carcinoma (including salivary gland primaries and metastases), or indeterminate and were correlated with surgical outcomes.

Results: A total of 183 cases of salivary gland FNAs followed by surgical excision have been identified (data analysis ongoing). There were 13 (7.1%) non-diagnostic FNAs, 100 cases diagnosed as B/LG (54.6%), 43 (23.5%) cases diagnosed as HG and 27 (14.8%) indeterminate cases. Thirteen of 27 (48.1%) indeterminate cases were basaloid neoplasms. For the 143 cases with an assigned risk category, 130 (90.9%) were correctly classified, while 13 (9.1%) were incorrectly classified. Six HG cases on surgical excision were incorrectly classified as B/LG on FNA (two salivary duct carcinomas, one adenoid cystic carcinoma, one metastatic squamous cell carcinoma, one carcinoma ex-pleomorhic adenoma, and one HG carcinoma not otherwise specified). In addition, three intermediate grade mucoepidermoid carcinomas were incorrectly classified as LG on FNA. Four cases incorrectly classified as HG on FNA were one intraductal low-grade cribriform cystadenocarcinoma, one epithelial-myoepithelial carcinoma, one Warthin tumor and one pleomorphic adenoma.
Conclusions: A risk-stratified approach to classifying salivary gland tumors on FNA is generally effective in distinguishing B/LG and HG tumors. However, basaloid neoplasms are difficult to risk stratify and are the leading source of an indeterminate FNA classification. Misclassification most frequently represents underdiagnosis on FNA with mucoepidermoid carcinoma the single most problematic entity.

429 MYB Immunohistochemical (IHC) Stain: Potential Role in Separating Adenoid Cystic Carcinoma (ACC) from Pleomorphic Adenoma (PA)

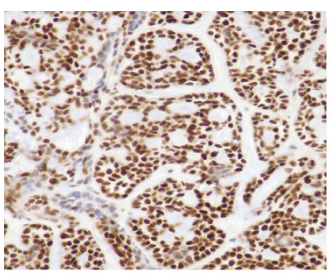
Andres Moon, Cynthia Cohen, Momin T Siddiqui. Emory University School of Medicine, Atlanta, GA.
Background: Basaloid tumors of the salivary gland include a group of benign and malignant tumors, comprising basal cell adenoma (BCA), basal cell adenocarcinoma, cellular PA and ACC. Although differentiation of these entities based on resected specimens may be unambiguous, rendering a diagnosis given a limited biopsy or fine needle aspiration (FNA) sample proves much more challenging. Activation of MYB by gene fusion has been found in salivary gland ACCs; therefore we investigated the utility of MYB IHC stain as a tool for distinguishing ACCs from other basaloid neoplasms.
Design: We selected 49 cases of ACC (11 FNA blocks [CB]), 38 histologic resections [HR]), 74 cases of PA (44 CB, 30 HR), and 18 cases of BCA (7 CB, 11 HR). IHC staining for MYB (Clone EP769Y, 1:100, Abcam, Cambridge, MA) was performed using a scoring system (0 to 3) to assess MYB nuclear staining intensity, with a score of ≥2 considered as positive.
Results: FNA CB showed 91% of ACCs (10 of 11 cases) as positive for MYB nuclear staining whereas 79% of ACCs (30 of 38) were positive in HR. Only 5% (2 of 44 cases) of PA were positive in FNA CB and 17% (5 of 30) in HR. For BCA, positive cases were comparable with ACC cases, showing 73% (8 of 11 cases) positivity in HR (p=0.674), with FNA CB showing a lower frequency of sensitivity (57% positive, 4 of 7 cases) compared to ACC (p=0.0907). Both ACC and BCA showed significantly higher mean staining intensity than PA in both FNA CB and HR (p<0.0001).

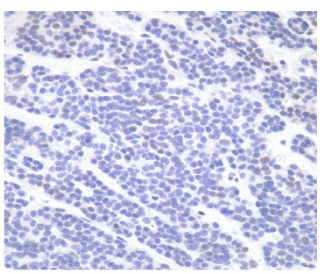
MYB staining pattern in CB

Tumor	Number of cases	Positive cases	% positive cases
ACC	11	10	91
PA	44	2	5
BCA	7	4	57

MYB staining pattern in HR

Tumor	Number of cases	Positive cases	% positive cases
ACC	38	30	79
PA	30	5	17
BCA	11	8	73


Positive MYB staining in ACC


Negative MYB staining in PA

Conclusions: The majority of ACC from both cytologic CB and HR cases are positive for nuclear MYB stain, whereas most cases of PA are negative. This difference in staining pattern was more evident in FNA CB than HR. MYB negative staining in PA may aid in differentiating it from ACC. Most BCA showed MYB positivity; it may prove to be a challenge to separating BCA from ACC, especially in limited cellular samples.

430 Anal Cytology: Institutional Review of Statistics and Histologic Correlation

Elizabeth Morency, Nazneen Fatima, Tracey Harbert, Dawn Heagley, Kruti Maniar, Ritu Nayar. Northwestern University, Chicago, IL.
Background: The incidence of anal cancer in the US is on the rise, especially in high-risk populations. The Anal Pap Test (APT) is advocated as a screening tool, in addition to digital rectal exam and high resolution anoscopy(HRA), however its efficacy in preventing anal cancer has not been demonstrated. We have a significant number of APT and herein review our experience.
Design: All APT cytology collected between January 2013 to June 2015 were studied and correlated with concurrent/follow up biopsy (bx), clinical and social history. HPV testing was not performed routinely with anal cytology until mid 2015. Internists and nurse practioners obtain APT and conventional anoscopy, not HRA, is performed by colorectal surgeons.
Results: A total of 1417 APT, from 1185 patients, prepared by ThinPrep methodology were retrieved. 90% were males and 10% females; ranging from 19-83 years; and 68% were HIV positive. APT results by Bethesda category were: 17.4% unsat, 27.9% NILM, 19.5% ASC-US, 24.1% LSIL, 3.6 % ASC-H and 7.5% HSIL. Bx correlation was available in 376/1417 cases displayed below with (%).

Bx/ Pap	Unsat (n=38)	NIL (n=73)	ASC-US (n=60)	LSIL (n=130)	ASC-H (n=14)	HSIL (n=61)
Neg	12 (31.6)	25 (34.2)	15 (25)	15 (11.5)	5 (35.7)	8 (13.1)
LSIL	22 (57.9)	42 (57.3)	38 (63.3)	85 (64.5)	7 (50)	22 (36.1)
HSIL	4 (10.5)	5 (6.8)	6 (10)	28 (21.5)	2 (14.3)	28 (45.9)
SCC	0	1 (1.4)	1 (1.7)	2 (1.5)	0	3 (4.9)

APT with ASC-US or higher had an 83.8% rate of bx proven disease (AIN1+). On APT-Bx correlation: 65.4% of LSIL had AIN1 and 50.8% HSIL had AIN2+. Sensitivity was even higher (92%) for detection of AIN2+. Overall test performance for detection of AIN2+ using any abnormal cytology result was- specificity 26%, NPV 92% and PPV 26%.
Conclusions: (1) Anal cytology had a high abnormal rate (54.7%) and sensitivity but low PPV and specificity for the detection of AIN. ASC-US comprised 36% of abnormal APT. (2) Correlation with histologic grade showed more HSIL on APT than bx. This differs from previous reports, however most of those used HRA. (3) Our high unsat rate indicates need for improved sampling, especially since 68.4% of these cases had SIL on follow up. Review of all unsat cases with SIL on bx, showed inadequacy in 80.8% and LSIL+ was found to have been missed by CT/MD in 19.2% suggesting any atypia warrants at least a diagnosis of ASC-US. (4) Further studies incorporating reflex hrHPV for ASC-US, which are now being routinely performed in house, are warranted to see the effect on improving detection of AIN/cancer.

431 Comparison of Molecular Platforms for Detection of HPV in FNA Biopsies of the Head and Neck

Zoltan Nagymanyoki, William Karlon, William C Faquin, Jeffrey F Krane, Neal Lindeman, Dimity Hall, Brenda J Sweeney, Britt-Marie Ljung, Ronald Balassanian, Annemieke van Zante. University of California, San Francisco, San Francisco, CA; Massachusetts General Hospital, Boston, MA; Brigham and Women's Hospital, Boston, MA; West Pacific Medical Laboratory, Santa Fe Springs, CA.
Background: High-risk HPV (HR-HPV) subtypes have been associated with squamous cell carcinoma (SCC) of the head and neck (H&N). HPV testing currently provides prognostic information and may guide therapy in the future. We compared 3 platforms currently available for cervical cancer screening (Cervista™, Aptima™ and Cobas™) for detection of HPV in SCC of the H&N.
Design: Fifteen fine needle aspiration (FNA) biopsy specimens from patients with SCC of the H&N were collected with rapid on site evaluation. One pass was rinsed into 9 ml of CytoLyt™ solution, divided into 3 equal aliquots, and transported to 3 academic clinical laboratories. Each laboratory used a different platform and tested all 15 samples. The results were reported based on the FDA approved cut-off values for cervical Pap testing.
Results: All 3 laboratories reported valid results for all 15 cases. Five of 15 cases were positive for high-risk HPV (type 16) on Cobas™ and Cervista™ platforms. Aptima™ identified 4 out of 5 high-risk HPV positive cases (type 16). In the one discrepant case where the Aptima™ result was negative, the Cobas™ viral CT value was high (40.2). Cobas™ and Cervista™ platforms had 100% concordance. Aptima™ had a Kappa value of 0.8421 when compared to the other two platforms.
Conclusions: Cervista™, Aptima™ and Cobas™ platforms are all suitable for HPV testing in SCC of the H&N. A single FNA pass is sufficient for detection of high risk HPV using molecular methodology. One discrepant result was found in comparing the 3 available platforms; this may be the result of borderline cellularity or a low level of viral E6/E7 RNA expression. Any of these three commercially available platforms is an alternative for laboratories that are not equipped to perform in situ hybridization for high risk HPV.

432 Utility of Immunohistochemistry and ETV6 (12p13) Gene Rearrangement in Identifying Mammary Analog Secretory Carcinoma of Salivary Gland among Previously Diagnosed Cases of Acinic Cell Carcinoma

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Background: Mammary analog secretory carcinoma (MASC) of salivary gland is a recently described entity with characteristic immunoprofile and *ETV6* (12p13) gene rearrangement. Before its initial description, it was generally diagnosed as acinic cell

carcinoma (ACCi). We evaluated immunoprofile and *ETV6* (12p13) gene rearrangement in cytological as well as surgical cases of previously diagnosed ACCi, in an attempt to identify any misclassified MASC.
Design: 15 cases (5 cytology with surgical follow up, 3 cytology only and 7 surgical only) of salivary gland ACCi diagnosed over a 13-year period were retrieved from our cytology and surgical pathology files. All cases were subjected to immunohistochemistry for S-100, mammaglobin, and GATA-3 (a panel that has been reported positive in cases of MASC) as well as fluorescence in situ hybridization (FISH) using a break apart probe for *ETV6* (12p13) gene. Archival cell and tissue blocks were used for FISH and immunohistochemical analysis.
Results: The patients' age ranged from 10 to 72 years (mean [49.3], median [53], males [5] and females [10]). Anatomic locations included left parotid (10), right parotid (4) and right neck (1). Of the 8 cytology cases only 1 (12.5%) was positive for S100, GATA-3 and mammaglobin and also demonstrated *ETV6* gene rearrangement (positive translocation). This case was reclassified as MASC. Of the remaining 7 cytology cases with negative immunoreactivity and lack of *ETV6* gene rearrangement, 5 had corresponding surgical follow up with similar results. Strong immunostaining for S100, GATA-3 and mammaglobin was present in only 1 (14.28%) of the 7 surgical cases. *ETV6* gene rearrangement characterized by 3' interstitial deletion was detected in this case and it was reclassified as MASC. Additional 6 surgical cases with negative immunoreactivity lacked *ETV6* gene rearrangement. Immunohistochemistry and *ETV6* gene rearrangement was useful in identifying 2 (13.3%) cases misclassified as ACCi.
Conclusions: Based on our case series, ACCi is infrequently misclassified. Characteristic immunoprofile (S100, GATA-3 and mammaglobin positivity) and *ETV6* gene rearrangement may prove useful in identifying cases of MASC, thereby preventing their misclassification as ACCi. Similar to the prior study by Pinto et al (Mod Pathol.2014;27(1):30-37), the presence of *ETV6* 3' interstitial deletion in one of our cases suggests that there may be additional *ETV6* related genetic alterations contributing to the pathogenesis of MASC.

433 Is There a Value for Age-Based Anal Cancer Screening in HIV-Infected Males?

Rana Naous, Lucinda Steele, Kamal K Khurana. SUNY Upstate Medical University, Syracuse, NY.
Background: The American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology recommend that cervical cancer screening should begin at age 21 with cytology alone, while cytology and HPV testing (cotesting) is advised for women aged 30 years or older. However, anal cancer screening lacks such guidelines. We compared the distribution of epithelial cell abnormalities in HIV-infected male population < 30 years old and ≥ 30 years old to assess if there were any significant difference in the two populations that may also provide a basis for age-based anal cancer screening guidelines in high risk males.
Design: Anal Pap tests in high risk (HIV infected) male patients between January 2009 and December 2014 were retrieved via computerized search. Patients were stratified by age into two groups Group 1 (<30 years old) and Group 2 (≥30 years old). Distribution of NILM cases and epithelial cell abnormalities in each group was evaluated and analyzed using Pearson's Chi square test.
Results: Out of a total of 902 cases of HIV infected males, 182 (20.2%) cases were unsatisfactory. Group 1 and 2 included 117 (12.9%) and 603 (66.8%) satisfactory cases, respectively. The distribution of NILM cases and epithelial cell abnormalities in the two groups is shown in table 1.

Diagnostic Category	Group 1 (< 30 years old)	Group 2 (≥30 years old)
NILM	35(29.9%)	265(43.9%)
ASCUS	28(23.9%)	151(25%)
ASC-H	0	5(0.8%)
LGSIL	47(40.2%)	129(21.4%)
HGSIL & LGSIL-HGNEX	7(5.9%)	53(8.8%)
Total	117(12.9%)	603(66.8%)

There was a statistically significant difference in the proportion of cases with NILM and epithelial cell abnormalities in the two groups (P< .001, pearson chi square test). LGSIL lesion was predominant in Group 1 (40.2% vs 21.4%) and LGSIL-High grade not excluded (HGNEX) and HGSIL was more common in group 2 (8.8% versus 5.9%).
Conclusions: Statistically significant distribution of cases between the two groups with predominance of LGSIL in young males (<30 years old) and HGSIL and LGSIL-HGNEX in older males (≥30 years old) suggests that there may also be a need for age-based guidelines for anal cancer screening in HIV infected males.

434 Detection of EGFR Mutations, ALK, and ROS1 Rearrangements in Cytological Specimens: An Institutional Experience

Rana Naous, Shengle Zhang, Kamal K Khurana. SUNY Upstate Medical University, Syracuse, NY.
Background: Molecular testing for *EGFR* mutations, *ALK*, and *ROS1* rearrangements in lung adenocarcinoma has become a model paradigm in targeted therapy.Cytology is first line of investigation for diagnosis of lung cancer. We sought to investigate the utility of molecular testing for *EGFR* mutations, *ALK*, and *ROS1* rearrangements in cytological specimens of primary or metastatic lung adenocarcinomas.
Design: A computerized search of all patients with primary or metastatic lung adenocarcinoma between September 2009 and August 2015 that underwent molecular testing for *EGFR* mutations, *ALK*, and *ROS1* rearrangements in cytologic specimens was performed. The specimen sites included mediastinal lymph nodes, lung, rib, parotid, submandibular gland, skin, and body fluid/effusions. *EGFR* mutations in exons 19 (del E746-A750) and 21 (L858R) were analyzed (Qiagen, Valencia, CA). Fluorescence in situ hybridization (FISH) studies for *ALK* and *ROS1* rearrangements were performed using dual-color Vysis LSI Break Apart Probe kit (Abbott Molecular, Downers Grove, IL). Cell blocks were routinely used for all molecular tests. Direct smears were used in cases of inadequate cell block material.
Results: Of 156 cases (FNA [139], pleural fluid [12], pericardial fluid [2], CSF [1], bronchial brushing [1], bronchioalveolar lavage [1]) tested for *EGFR* mutations, 5 cases (3.2%) were rejected due to insufficient material and 10 cases (6.6%) tested positive for either exon19 (6) or 21 (4) mutations. Among 144 cases (FNA [125], pleural fluid [15], pericardial fluid [1], bronchial brushing [2], bronchial wash [1]) tested for *ALK* rearrangement, 3 cases (2.1%) were rejected due to insufficient cellularity, and 4 cases (2.8%) tested positive. All of the 40 cases (FNA [35], pleural fluid [4], bronchial brushing [1]) submitted for *ROS1* analysis showed absence of *ROS1* rearrangement (0%). Table 1 shows number of cases where cell blocks or smears were used in each of the molecular tests.

Test	Cell block	Smear	Total
EGFR mutation	105 (69.5)	46 (30.5%)	151
ALK rearrangement	120 (85.1%)	21 (14.9%)	141
ROS1 rearrangement	38 (95%)	2 (5%)	40

Conclusions: Cytologic specimens yield adequate material (adequacy rate ≥ 96.8%) for molecular testing of *EGFR* mutations, *ALK*, and *ROS1* rearrangements. The detection rates of these oncogenic drivers (6.6% for *EGFR* mutations, 2.8% for *ALK* rearrangement and 0% for *ROS1* rearrangement) in our series are within the range of reported rates in primary or metastatic lung adenocarcinomas.

435 A Comparison of Automated Digital Image Analysis (DIA) and Manual Count of Camera-Captured Images in Calculating Ki-67 Proliferation Index (PI) in Cytologic Samples from Pancreatic Neuroendocrine Neoplasms (PanNENs)

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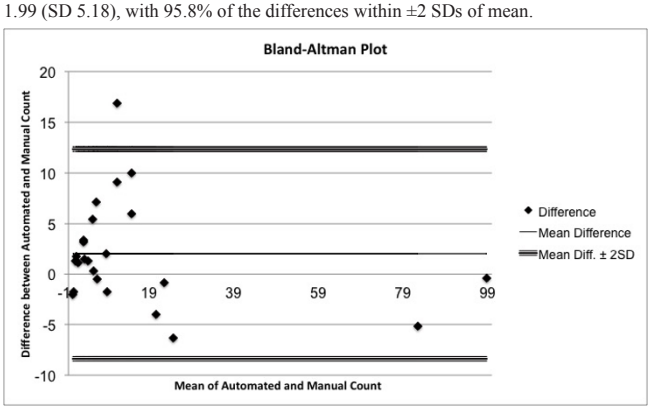
Background: In 2010 the WHO revised the grading scheme of PanNENs to encompass Ki-67 PI. Of current PI quantification methodologies, manual counting of camera-captured/printed images (MCI) has proven to be the most accurate and reproducible (PMID: 25412850); however, automated counting is also widely used.
Design: We quantified the Ki-67 PI in cell blocks from 24 PanNENs using automated count with DIA software and compared the results to those generated by MCI. For DIA, whole slides were scanned using Aperio ImageScope. For each slide, 3 areas of greatest Ki-67 positivity ("hot spots") were selected by a pathologist, and PI was calculated using DIA software algorithm. For MCI, a microscope was used to identify 3 designated hot spots, which were photographed, printed in color, and the Ki-67-positive and negative tumor nuclei were counted to calculate PI.
Results: DIA and MCI resulted in concordant grades in 21/24 cases (88%), with Pearson's R-value of 0.98 (p<.00001). On average, 1256 nuclei were counted/sample by DIA (range 230-3337). On average, 1037 nuclei were counted/sample by MCI (range 455-1457). There were 3 discordant cases: 2 upgraded from grade 1 to 2 on DIA, and 1 downgraded from grade 3 to 2 on DIA, with a difference in PI of 3.2, 3.34, and 4, respectively (each within the categorical border zone).

		MCI (n)				
	Grade	**1**	**2**	**3**	Total	
DIA (n)	**1**	5	0	0	5	R=0.98
	2	2	12	1	15	p<.00001
	3	0	0	4	4	
	Total	7	12	5	24	

The Bland-Altman plot for DIA and MCI comparison showed a mean difference of 1.99 (SD 5.18), with 95.8% of the differences within ±2 SDs of mean.



Conclusions: In calculating Ki-67 PI, DIA and MCI correlated significantly, producing concordant grades in the majority of cases. These results suggest that automated DIA is a promising technique for quantifying Ki-67 PI in cytologic samples from PanNENs. However, it is important that tumor hot spots be preferably selected by a pathologist, especially on cytology samples, where the risk of non-tumoral contaminants (lymphocytes, pigmented macrophages) is greatest.

436 Diagnostic Accuracy and False Negative Rate of EBUS-TBNA in Detection of Well Differentiated Neuroendocrine Tumors of the Lung

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Background: Endobronchial ultrasound guided transbronchial needle aspiration (EBUS-TBNA) is being increasingly used to diagnose lung cancers including neuroendocrine tumors of the lung. The objective of this study is to determine the accuracy of EBUS-TBNA in recognition of neuroendocrine tumors by conducting cytological to histological correlation.

Design: A computerized search of the Copath database was performed and all cases which had a diagnosis of carcinoid / neuroendocrine tumor on surgical resection were identified. All correlating EBUS-TBNA cytology report diagnoses were reviewed retrospectively. Sensitivity, positive predictive value and false negative rates of EBUS-TBNA were calculated.

Results: From 2010-2015, 38 cases were diagnosed as carcinoid or neuroendocrine carcinoma after surgical resection. Of the 31 cases which had a concordant cytological diagnosis of carcinoid, 27 were typical carcinoid while 4 were atypical carcinoid. Of the 7 cases which had a discordant cytological diagnosis on EBUS-TBNA, 6 cases were reported negative on EBUS-TBNA, while in one case which was diagnosed as carcinoid on EBUS-TBNA, the diagnosis was upgraded to large cell neuroendocrine carcinoma (LNEC) on histology. Diagnostic sensitivity of EBUS-TBNA was calculated to be 0.82 (95% CI 0.66 – 0.93), positive predictive value of EBUS-TBNA was 1 (95% CI 0.89 – 1), and false negative rate was 0.18. Diagnostic accuracy was found to be 82%. The commonest cause of negative cytology was determined to be a paucicellular sample and was found in 6 of 7 cases, while the case which received an upgraded diagnosis on resection had large atypical cells on cytology.

Conclusions: EBUS-TBNA has a high sensitivity, positive predictive value and accuracy when used for the diagnosis of well differentiated neuroendocrine tumors of the lung. Although the false negative rate was concerning, there is potential for improvement by better sampling techniques, for which further studies may be needed. While, examination of surgical excisions still seems necessary for classification, EBUS-TBNA appears reliable for diagnosing these neoplasms when the lesion is well sampled.

437 Improved Detection of CIN 2+ Lesions by the Becton Dickinson Focal Point™ GS Slide Profiler No Further Review Technology

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Background: Background: The use of Computer Assisted Screening (CAS) in cervical screening is well documented in the USA and Europe. MAVARIC, a UK trial, compared automation-assisted reading with manual reading of cervical cytology samples for detection of CIN2+ and concluded that the "significantly reduced sensitivity of automated reading, combined with uncertainty over cost-effectiveness, suggests no justification at present to recommend its introduction. The reliability of 'no further review' warrants further consideration as a means of saving staff time."

The objective of this study was to evaluate the Becton Dickinson (BD) FocalPoint™ GS "No Further Review" (FocalPoint™) slide reporting technology as an alternative for manual primary cytology screening in the Cervical Screening Programme within Wales, UK.

Design: This health technology assessment, designated as **CAESAR** (**C**omputer **A**ssisted **E**valuation – **S**creening **A**nd **R**eporting), is a prospective, multi-centre (N=4) randomised controlled trial conducted between 2006 and 2011. Women (n=45,317) aged 20-64 undergoing primary screening in Wales in the UK were randomly assigned to manual arm only or paired arm (manual and automated) using the FocalPoint™ automated technology.

Slides were scanned using the FocalPoint™ technology and manually primary screened. A comparative assessment was then carried out between the slides categorised as No Further Review (NFR) with negative Quality Assurance screen and slides that were manually screened and reported as negative

Results: Comparing 3 year interval CIN 2+ cases that resulted from samples reported as NFR (n = 8130) and manual screening samples reported as negative (n = 93473) over the same period, the proportion of cases in the NFR cohort was statistically significantly lower.

When the CIN 2+ interval outcome cases were categorised into cancers and pre-cancers, the interval cancer prevalence at 3 years between NFR and manually screened samples was similar; however, the interval pre-cancer prevalence for FocalPoint™ NFR was half that of manual screening – indicating that the NFR technology was at least equivalent to manual screening for detecting CIN.

Conclusions: The FocalPoint™ CAS technology has a number of potential quality and throughput benefits to support and enhance performance within the cervical screening programme.

438 A Multiinstitutional Interobserver Variability (IOV) Study Interpreting Thyroid FNA using the Bethesda System for Classification of Thyroid Cytology (TBSCTC) - A Focus on Atypical Cells of Undetermined Significance (AUS)/ Follicular Lesion of Undetermined Significance (FLUS)

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Background: The goal of TBSCTC was to standardize classification to allow for a uniform reporting system for thyroid FNA. However, the AUS/ FLUS category is controversial, and the reported atypia rate in thyroid cytology has varied widely in the literature. The aim of this study was to assess reproducibility of the AUS/FLUS category and IOV among cytopathologists from the CAP cytopathology committee.

Design: Each of the 7 participating cytopathologists provided 11 consecutive thyroid FNA cases: atypical (AUS/FLUS) (5) and non-atypical (6) (benign, suspicious for follicular neoplasm and suspicious for malignancy). Cases were reviewed by all participants and diagnosed using TBSCTC. A nonlinear -mixed model was used to analyze the attributes using SAS 9.2 (SAS Institute; Cary NC).

Results: There were 486 reviews of 75 cases by 7 cytopathologists. The overall concordance rate was 44.7% (217/486). Cellular adequacy and diagnosis were significantly associated with the concordance rate. Seventy eight of 125 (62.4%) benign cases were classified as benign by the reviewers and 21% of the benign cases were called AUS/FLUS. A third of the AUS/FLUS cases were called benign while a third correlated with the original pathology report and were classified as AUS/FLUS and 28.2% of the AUS/FLUS cases were classified as suspicious for neoplasia/ suspicious for malignancy. There was significantly higher concordance of benign cases with macrofollicular/flat sheet architecture ($p < .001$) and conversely, "Suspicious for neoplasm/malignancy" significantly correlated with microfollicular pattern.

Conclusions: When pathologists from different institutions shared their slides, concordance was high for specimens with adequate cellularity and those that were clearly benign but thresholds varied for the other indeterminate categories. In cases where an AUS/FLUS diagnosis is entertained a second opinion may aid in rendering a more definitive diagnosis. This may reduce the overall atypia rate, which is especially important as each category in TBSRTC is associated with different rate of malignancy and different types of management.

439 Indefinite to Positive and Indefinite to Negative Ratios as Potential Quality Measures for Urine Cytology

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Background: In gynecologic cytology, the ratio of ASC-US to LSIL, in combination with high-risk HPV rate, is a commonly used indicator of pathologists' diagnostic precision. While no such standard currently exists in non-gynecologic cytology, there have been efforts to evaluate thyroid cytology via a ratio of atypia of undetermined significance to malignancy. In light of the ongoing movement toward a standardized nomenclature (Paris System) for urine cytology, it is worthwhile to evaluate whether similar measures could be used to assess urinary cytology.

Design: Urine cytology cases, totaling 821 cases, were identified at our institution from September 2012 to September 2015. Rates of negative, atypical, suspicious, and positive for high-grade urothelial carcinoma (HGUC) diagnoses were assessed. Follow-up data for indefinite (atypical/suspicious) cases was reviewed and indefinite to negative (I/N) and indefinite to positive (I/P) ratios were assessed for each pathologist and the lab as a whole.

Results: The 821 cases were roughly evenly distributed between 4 pathologists. The diagnosis rates were 83.8% negative (range: 81.4-86.2%), 11.7% atypical (range: 8.1-15.3%), 1.9% suspicious (range: 0.7-3.3%), and 2.6% positive (range: 1.6-3.7%). The I/N ratio was 0.16 (range: 0.12-0.20) and the I/P ratio was 5.3 (range: 2.7-9.3). Definitive follow-up data was available for 55 indefinite cases (45 atypical and 10 suspicious), of which 40% had HGUC. Data is shown in table 1.

	Lab Average	Pathologist 1	Pathologist 2	Pathologist 3	Pathologist 4
Negative (%)	83.8	83.7	86.2	82.0	81.4
Atypical (%)	11.7	13.1	8.1	15.3	13.1
Suspicious (%)	1.9	1.6	2.0	0.7	3.3
Positive (%)	2.6	1.6	3.7	2.0	2.2
I/N ratio	0.16	0.18	0.12	0.20	0.20
I/P ratio	5.3	9.3	2.7	8.0	7.5
Positive F/U (%)	40	0	38	56	60

Conclusions: There was substantial variation in the rates of indefinite diagnoses between pathologists; however, these rates correlated imperfectly with clinical follow-up. The indefinite rate may be a useful indicator for evaluating the level of diagnostic confidence of an individual pathologist, but does not provide information as to whether an abnormally high indefinite rate is due to overcalling negative findings or under-calling positive findings. The combination of I/N and I/P ratios could provide this information to an individual pathologist and comparison of individual data to lab, or ideally national, benchmarks could be valuable as a quality measure.

440 An Emerging Role for Cytopathology in Precision Oncology

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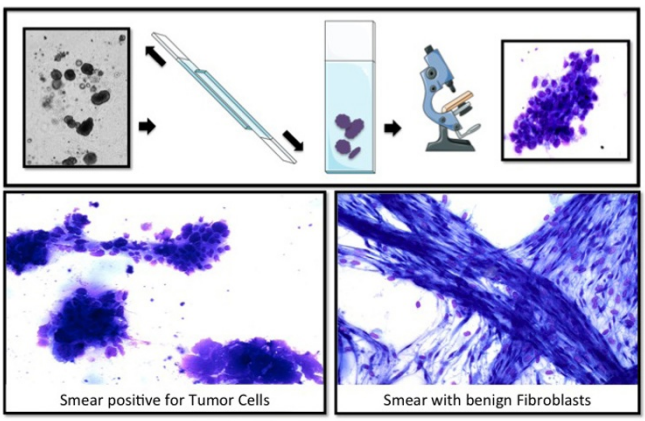
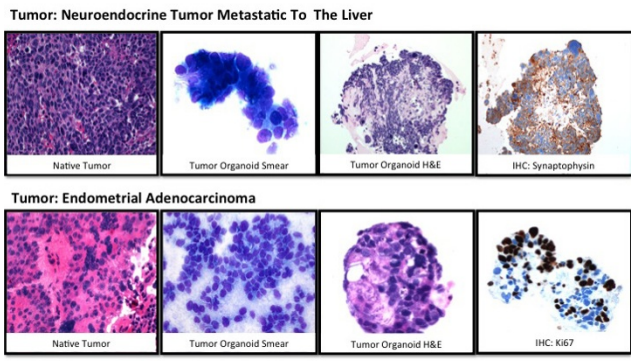
Background: Precision Medicine is an emerging field in medicine for disease prevention and treatment that takes into account the individual variability in genes, environment, and lifestyle for each person. As part of the Institute for Precision Medicine at Weill Cornell Medical College we developed a program to grow patient-derived tumor organoids for drug screenings, tailoring treatment strategies and as models for studying drug resistance. One of the limiting factors in growing primary tumor samples is the "contamination" of these samples by benign cells from adjoining tissues.

Design: Patient derived tumor organoids are generated from freshly collected tumor biopsies. As an initial screening platform, cytology smears from cultured organoids are prepared and evaluated by a cytopathologist to ensure that only tumor organoids and not benign cells are further cultured.



Tumor organoids are additionally characterized by using routine histology and ancillary testing such as IHC and/or FISH.



Immunofluorescence has been used to visualize a specific protein and next generation sequencing offers genomic characterization.

Results: We established different platforms for the characterization and validation of patient derived tumor organoids. In our program, cytopathology preparations have become a very efficient and cost-effective screening tool to confirm tumor organoid cultures and are an absolute requirement to ensure good culture quality. Molecular characterization and validation of cytopathology confirmed tumor organoids show good concordance between native tumor tissue and matching tumor organoid.

Conclusions: Patient derived cancer cell cultures represent a promising area in personalized medicine, to test drug sensitivity and to study drug resistance. We developed platforms for the characterization and validation of patient-derived tumor organoids and illustrate the role of cytology as a cost effective and powerful quality control measure.

441 Fine-Needle Aspiration of Thyroid Malignancies Arising in Graves' Disease: A Diagnostic Challenge?

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Background: Thyroid fine-needle aspiration (FNA) in the setting of Graves' disease (GD) can be diagnostically challenging because of cytomorphological overlap between the changes of GD, antithyroid treatments effects (ATE), and papillary thyroid carcinoma (PTC). We analyzed a large FNA series of 155 thyroid nodules in GD, and correlated with histological follow-up.

Design: A 17-year retrospective search from two large academic medical centers was performed. The Bethesda System for Reporting Thyroid Cytopathology was used to classify FNA reports.

Results: 155 FNAs from 102 patients with GD were identified, and reported as follows: 20 (12.9%) non-diagnostic; 63 (40.6%) Benign; 26 (16.8%) AUS/FLUS; 22 (14.2%) FN/SFN; 13 (8.4%) Suspicious for Malignancy (SM); 11 (7.1%) Malignant. Twenty-six patients had thyroid malignancy, mostly PTC, in the corresponding nodule(s) on histological follow-up, while 13 patients had incidental papillary microcarcinomas. The risk of malignancy for each cytologic diagnostic group was as follows: non-diagnostic, 0/20 (0%); Benign, 2/63 (3.2%); AUS/FLUS, 5/26 (19.2%); FN/SFN, 4/22 (18.2%); SM, 9/13 (69.2%); and Malignant, 11/11 (100%). Four cases of benign nodules, including 3 associated with ATE, were interpreted as SM. Two cases of follicular variant of PTC were diagnosed as "FN/SFN", and 2 cases of PTC other than follicular variant were diagnosed as benign and as AUS/FLUS, respectively.

Conclusions: The rate of benign cytologic diagnoses of thyroid nodules is lower in a GD population than in a non-GD population, and this is reflected in a higher rate of indeterminate cytologic diagnoses in the former. This supports that thyroid malignancy may be more difficult to accurately diagnose and to distinguish from benign lesions in GD patients, and both clinicians and pathologists should be aware of this diagnostic shift.

442 Next Generation Sequencing Adds Value to the Pre-Operative Diagnosis of Pancreatic Cysts

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Background: The diagnosis of a pancreatic cyst as mucinous and high-risk dictates the need for surgery. Molecular analysis of aspirated pancreatic cyst fluid (PCF) can provide valuable information that may not be obtained by CEA analysis or cytology, thus adding diagnostic value to the preoperative diagnosis and patient management.

Design: All patients with molecular analysis of PCF between 03/2013 and 06/2015 were reviewed along with associated pathology, imaging, and clinical follow-up. Molecular testing was performed using MGH's patented anchored multiplex PCR next generation sequencing (NGS) platform, which sequences numerous exons of 39 genes with established links to malignancy. Performance of NGS was calculated using final outcome determined by pathology or conventional clinical methods of imaging, CEA, and cytology.

Results: The study cohort consisted of 125 PCF from 114 patients (Table 1). NGS was successful in 96% of the cases, and was positive in 56% of cases (Table 2). The majority of mutations were *KRAS* (49%) or *GNAS* (23%), which supported a mucinous etiology in 21 cases lacking other evidence of a mucinous tumor. TP53 mutation was seen in 4 cases diagnosed as adenocarcinoma. *CDKN2A* was seen in 10 cases, 8 of which were diagnosed as malignancy. In addition, VHL mutations were seen in two cysts thought to be IPMN by imaging. The presence of two or more mutations was significantly correlated with high-grade atypia or malignancy (RR=3.2; $p<0.001$). In the 25 cases with resection or core biopsy, a KRAS or GNAS mutation had a 74% sensitivity and a 83% specificity for a mucinous neoplasm and GNAS mutation was 37% sensitive but 100% specific for IPMN.

Conclusions: Molecular analysis of PCF provides valuable information in addition to CEA and cytology for accurate pre-operative diagnosis. Mutations in *KRAS* or *GNAS* define a mucinous neoplasm whereas GNAS is highly specific for IPMN. Late mutations in progression to malignancy support a high-risk cyst requiring surgery.

Table 1: Patient and Cyst Characteristics	
Number of samples	125
Number of patients	114
Median age (years)	68
% Female	53
Low Risk (no high grade atypia or high risk imaging)	103
High Risk (high grade atypia and/or high risk imaging)	22
Mucinous (CEA >192 ng/ml and/or mucinous cytology)	55
Non-mucinous (no evidence of mucin by CEA and cytology)	70

Table 2: Mutations Found in PCF Specimens	
GNAS	27/120(23%)
KRAS	59/120(49%)
TP53	4/120(3%)
CDKN2A	10/120(8%)
Other	7/120(6%)

443 Evaluation of Flow Cytometry Results from Serial Lymph Node Specimens Shows Equally Robust Results for FNA and Core Needle Biopsy Specimens in Comparison to Open Biopsy Specimens

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Background: Flow cytometry (FC) of lymph node (LN) tissue is an important diagnostic tool. Increasingly, LN tissue is procured by minimally invasive techniques, namely fine needle aspiration (FNA) and core needle biopsy (CNB), as opposed to open biopsy. FNA and NCB result in smaller samples with fewer cells, which theoretically might negatively affect the sensitivity of FC, when compared to evaluation of open biopsy specimens.

Design: We searched our FC records, spanning a period since installation of our current FC analysis software (10/2012-9/2015; 3 yrs) for patients who had serial specimens from the same LN obtained within 6 months of each other. We recorded the type of sample (FNA, excision, etc.), the number of B cells and T cells analyzed, and the kappa:lambda ratio (K:L). We recorded the CD4:CD8 ratio (4:8) when these antigens were assessed on both samples. We analyzed the data to determine if the number of cells correlated with the procedure, and whether differences in the ratio of B cells to T cells (B:T), K:L, and/or CD4:CD8 existed between serial specimens.

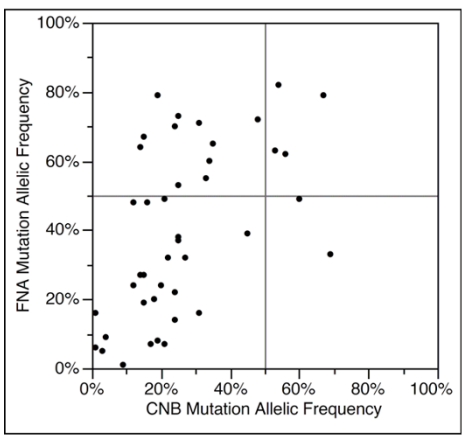
Results: 27 unique patients had two serial LN specimens analyzed by FC. For 21 patients the initial specimen was an FNA; in the remaining 6 it was a CNB. For 25 patients, the second specimen was an excisional biopsy; for the remaining 2 it was a repeat FNA. The average interval between the two procedures was 22 days. Open specimens did yield more cells for analysis than FNA and CNB specimens, though the difference was not statistically significant. FNA provided a larger number of cells for evaluation than CNB. There was strong correlation between the B:T, K:L, and 4:8 obtained from the initial minimally invasive procedure and the subsequent open procedure.
Conclusions: Our data show that FC of small specimens obtained via minimally invasive techniques does not differ significantly from the data obtained from analysis of larger specimens obtained via open surgery. This supports the diagnostic utility of FNA and CNB. While some patients undergoing FNA and/or CNB of LN will still need to undergo a subsequent open biopsy to establish diagnosis, possibly due to the need for a greater amount of tissue for histology or to obtain tissue for ancillary testing, this data suggests that open LN biopsy is not needed for purposes of obtaining better FC results. In addition, our data suggest that when a patient undergoes both FNA and CNB, better FC results might be expected from the FNA specimen.

444 Comparing Next-Generation Sequencing in Concurrently Acquired Cytology Fine Needle Aspirations and Surgical Core Needle Biopsies of Solid Organ Malignancies

Sinchita Roy Chowdhuri, Rajesh Singh, Jawad Manekia, Bedia A Barkoh, Hui Yao, Savitri Krishnamurthy, L Jeffrey Medeiros, Gregg Staerkel, John M Stewart, Rajyalakshmi Luthra. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: Next-generation sequencing (NGS) is being increasingly used to direct personalized cancer therapy for solid organ malignancies. A large fraction of these diagnoses are established using small tissue biopsy samples, which include fine needle aspiration (FNA) and core needle biopsy (CNB) specimens. In this study we retrospectively reviewed concurrently acquired FNA and CNB samples to compare cellular adequacy and the results of NGS mutational analysis.
Design: A retrospective study of 24 paired consecutive FNA and CNB samples from CT-guided procedures performed at our institution, meeting a tumor fraction threshold of >20%, were analyzed by NGS. DNA was extracted from circled tumor-rich areas of FNA direct smears (Diff-Quik and Papanicolaou stained) and formalin-fixed paraffin-embedded unstained tissue sections prepared from CNB paraffin blocks. The cases were analyzed by NGS using the Ion Torrent PGM (Life Technologies, CA) and the Ampliseq Cancer Hotspot v2 panel.
Results: The overall DNA yield from FNA smears was significantly lower than that obtained from CNB specimens (p=0.01); however, the estimated tumor fraction was significantly higher in FNA smears than CNB samples (p=0.003).

		FNA	CNB
Tumor Fraction	Mean	54%	39%
	Median	60%	30%
	Range	25-90%	20-70%
DNA Yield	Mean	6.6 ng/ul	17.5 ng/ul
	Median	3.6 ng/ul	12.9 ng/ul
	Range	0.36-21 ng/ul	0.27-55 ng/ul

All somatic mutations detected in CNB samples were detected in FNA samples. The normalized average amplicon coverage for all 50 genes analyzed, as well as that of selected genes of relevance (*BRAF, KRAS, NRAS, HRAS, EGFR, PIK3CA, KIT, PTEN*, and *TP53*) was higher in the FNAs than the paired CNBs (p=0.025 and 0.014 respectively). In addition, the FNA samples had lower numbers of underperforming amplicons and higher overall mutation allelic frequencies than CNB samples.

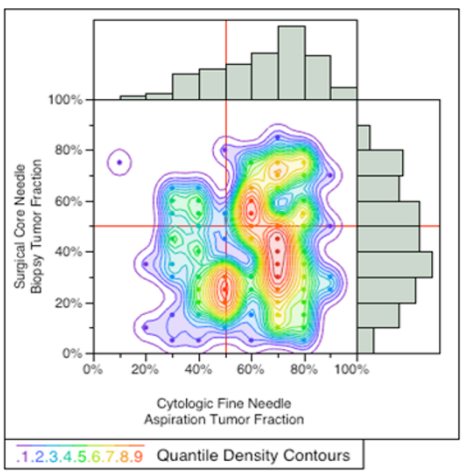


Conclusions: Cytology FNA samples provide an adequate tumor source for NGS analysis. Despite lower DNA yield, FNA smears generally provide higher quality DNA than concurrent CNB specimens as shown by higher mutation allelic frequencies, better amplicon coverage, and lower numbers of failed amplicons.

445 Morphologic Assessment of Cellularity and Tumor Fraction in Concurrently Acquired Cytology Fine Needle Aspiration and Surgical Core Needle Biopsy for Molecular Analysis

Sinchita Roy Chowdhuri, Hui Chen, Gregg Staerkel, John M Stewart. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: In an era of targeted therapy it is critical to be able to utilize small tissue samples, including fine needle aspiration (FNA) and core needle biopsy (CNB) specimens, for molecular testing. Although several studies have demonstrated the utility of cytology specimens for molecular analysis, there has been some controversy regarding the adequacy and suitability of FNA specimens and in most institutions a CNB is preferred over an FNA sample for molecular testing. In this study we retrospectively reviewed concurrent FNA and CNB samples comparing overall cellularity and tumor fraction in assessing adequacy for molecular testing.
Design: We evaluated 100 consecutive concurrent malignant FNA and CNB samples from image guided procedures performed at our institution. The samples were evaluated by light microscopy and scored for overall cellularity (high, moderate, and low) and tumor fraction. FNA samples included direct smears for all cases with additional cell block preparations in a subset of cases (n=50). Smears and cell blocks were scored separately and an overall tumor fraction was selected for each case. CNBs were scored based on H&E stained sections from 2 levels.
Results: Overall cellularity of the 100 cases reviewed was as follows: FNA smears, high: 29%; moderate: 37%; low: 34%, *versus* FNA cell blocks high: 34%; moderate: 42%; low: 24%, *versus* CNBs high: 15%; moderate: 58%; low: 27%. FNAs showing high cellularity with corresponding low cellularity CNBs were generally metastatic tumors. Neuroendocrine carcinomas and poorly differentiated carcinomas typically had higher cellularity on FNAs. FNA samples also had an inherently higher estimated tumor fraction than concurrent CNBs, with 88% of FNA samples having 40% or higher tumor fraction as compared to 54% of CNBs.

Tumor Fraction	FNA	CNB
<20%	1	14
20-39%	11	32
40-59%	24	30
60-79%	44	21
≥80%	20	3



Conclusions: Cytology FNA samples provide an adequate tumor source for ancillary studies and frequently provide superior cellularity and tumor fraction when compared to concurrent CNBs.

446 GATA3 Expression in Malignant Mesothelioma Is a Potential Pitfall in Effusion Cytology

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Background: GATA3 is a relatively new immunohistochemical marker expressed in breast and urothelial carcinoma, but is not specific to either. An early histologic study reported GATA3 positivity in approximately 50% of malignant mesotheliomas (MM). However, GATA3 has not been well studied in malignant effusion samples other than from breast carcinoma. We describe the nature of GATA3 staining in malignant and benign mesothelial effusions and potential mimics.
Design: Archival slides and cell blocks from 48 cytology effusion specimens were retrieved including 27 MM, 7 breast carcinomas (BC), 3 squamous cell carcinomas (SQCC) and 11 benign effusions. All diagnoses of MM were confirmed by tissue biopsy, and all benign effusions were from patients without history or follow-up diagnosis of cancer. GATA3 (L50-823; Biocare) immunohistochemistry (IHC) was performed on 5 µm sections from each cell block. Original slides, including other available IHC, were reviewed to characterize the cell populations present. GATA3 was scored qualitatively (0, 1+, 2+ or 3+; internal control T-cells were considered 2+) and quantitatively (0; 1-25%; 26-50%; 51-75%; >75% of cells). In cell blocks containing both carcinoma and benign mesothelial cells, each cell type was scored separately, if possible.
Results: GATA3 was positive in 7/7 (100%) BC, 24/27 (89%) MM, and 0/3 (0%) SQCC. In all BC, GATA3 positivity was strong (3+) and diffuse (>75%). GATA3 positivity in MM was variable. Moderate (2+) to strong (3+) staining was seen in 70% of cases (11

and 8 cases, respectively), but the range of cells staining in these cases varied from 26 to >75%. Five (19%) MM showed only focal/weak staining, and 3 were completely negative. Only 2/19 (11%) cell blocks (carcinoma and benign effusions) contained definite benign mesothelial cells that showed weak and focal staining.
Conclusions: The findings of this study suggest that GATA3 is positive in the majority of MM effusions, however the intensity and extent of staining is variable. Since some MM cases exhibited strong and diffuse GATA3 positivity, this may represent a potential pitfall when this marker is used in cell block specimens where the differential diagnosis includes breast or urothelial carcinoma. GATA3 does not appear to be more than weakly/focally expressed by benign mesothelial cells; therefore more than weak/focal GATA3 staining (intensity equal or greater to T-cells) may be a useful marker of malignancy in effusions suspicious for MM.

447 Applying Strict Cytomorphologic Criteria and Minimizing Interobserver Variability in Diagnosing "Atypia of Uncertain Significance" Increases the Positive Predictive Value of the Afirma GEC Assay in Thyroid Fine Needle Aspiration

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Background: Fine needle aspiration biopsy (FNAB) of thyroid nodules is limited in that a number of biopsies will be diagnostically inconclusive. The Afirma Gene Expression Classifier (Afirma GEC) stratifies patients into a "benign" or "suspicious" category associated with a "risk of malignancy" in patients whom a diagnosis of "Atypia of Undetermined Significance" (AUS) was rendered. Several studies have examined negative and positive predictive values (PPV) of the GEC test; however the role of interobserver variability and adherence to strict diagnostic criteria for the AUS category in influencing the overall value of a Afirma GEC result has not been assessed.
Design: A retrospective analysis of all cases of ultrasound-guided FNAB for a thyroid nodule in calendar year 2014 was performed. Each nodule had a minimum of 2 FNAB passes performed by a single cytopathologist under ultrasound guidance. The specimen was immediately assessed for adequacy and the presence/absence of atypia by strict diagnostic criteria. Patients with atypical features underwent 2 additional passes for Afirma GEC.
Results: 410 thyroid nodules were sampled from 235 patients (37 male, median age 61; 198 female, median age 60). 352 (86%) were classified as benign, 26 (6%) classified as Papillary Thyroid Carcinoma, and 31 (7%) classified as atypical (AUS) by a single cytopathologist. 1 was non-diagnostic. Afirma GEC was performed on 16 of the 31 AUS nodules and the remaining 15 underwent excision due to a very high suspicion for malignancy. Of the 16 in which Afirma GEC was performed, 8 were categorized as benign and 8 suspicious for malignancy by GEC. The 8 benign nodules were managed with routine follow-up. The 8 GEC-suspicious underwent excision yielding 1 Papillary Thyroid Carcinoma, 6 Follicular Variant of Papillary Carcinoma, and 1 adenomatoid nodule, yielding a PPV of 87.5%.
Conclusions: The PPV of a suspicious Afirma GEC result is higher in our laboratory than that seen in the reported literature. In preselecting through strict diagnostic criteria those patients in which the GEC is performed and minimizing interobserver variability, we have increased the PPV of a suspicious GEC result and minimized unnecessary molecular testing.

448 A Series of Clear Cell Papillary Renal Cell Carcinoma Sampled by Aspiration Cytology

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Background: Clear cell papillary renal cell carcinoma (CCPRCC) is a histomorphologically, immunohistochemically, and molecularly distinctive entity recently adopted by the Vancouver and new WHO Classifications of renal tumors. An emerging consensus documents its characteristically indolent course, emphasizing its separation from conventional clear cell RCC for treatment planning. CCPRCC features in aspiration cytology have not been systematically studied and reported.
Design: We retrospectively identified a series of cases by institutional database searches of renal cytology specimens diagnosed with clear cell and papillary features, reviewing corresponding case materials (aspirate smears, touch preps, cell block material, and any core biopsy or subsequent surgical excisions, &/or IHC) to identify cases of CCPRCC. Clinicopathologic and cytologic features identified were tabulated.
Results: Five cases of CCPRCC with cytopathologic material (2 aspirate smears, 1 cell block, 3 touch preps, and 5 cores) were identified from 3 females and 2 males, aged 34-70, 2 with end stage renal disease, sampling lesions from 1.8 to 11cm. Original diagnoses considered ranged from atypical cyst lining cells, to angiomyolipoma, to clear cell RCC, to CCPRCC on two most recent cases. The aspiration smears and touch preparations showed scant cellularity, with scattered sheets and clusters of small bland epithelial cells (quite smaller than admixed renal tubular cells) with optically clear cytoplasm, conspicuously lacking vacuolation, and small nuclei, apparent on both Pap and Diff-Quik stains. Two examples showed admixed foamy histiocytes. Cell block and cores showed cyst walls, including examples with prominent myomatous stroma, lined by diagnostic low grade epithelium with inverse polarization. Two cases were treated by nephrectomy, one additional with partial nephrectomy, one currently on surveillance and one is lost to follow-up.
Conclusions: CCPRCC is a recently categorized renal neoplasm that should be considered in the differential diagnosis of cytologic specimens showing bland cells with clear cytoplasm and low grade nuclei, suggestion of papillary architecture, and/or smooth muscle rich stromal fragments. While there may be cytologic overlap with papillary and clear cell RCCs, these suggestive features may trigger further IHC workup (CK7 & CAIX positivity) leading to correct classification with impact on treatment planning.

449 Accuracy of Grading Pancreatic Neuroendocrine Tumors on Fine Needle Aspiration Samples

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Background: The initial diagnosis of pancreatic neuroendocrine tumors (pNETs) is commonly made on the basis of cytologic material obtained by endoscopic ultrasound-guided fine needle aspiration biopsy (EUS-FNA). Since tumor grade has been shown to correlate strongly with patient outcome, there has been considerable interest in grading these tumors preoperatively. In this study, we assess the accuracy of grading pNETs on the limited material obtained by EUS-FNA.
Design: A pathology database search from 1/2008 to 8/2015 yielded 31 pancreatic neuroendocrine tumors that had pre-operative EUS-FNA and resection specimens. Pre-operative grade was assessed on cell block material on the basis of mitotic index (MI), Ki-67 proliferation index (MIB-1 clone; 1:200 dilution; Leica Biosystems Inc., Buffalo Grove, IL), and presence/absence of necrosis. MI was determined by counting mitotic figures in 50 high power fields (HPF) and reporting per 10 HPF. KI-67 proliferation index was determined by counting 500 cells in the most active areas. In cases with fewer than 500 cells, proliferation index was determined by counting all neuroendocrine cells present. Grade was assigned as follows: grade 1 MI <2, Ki-67 ≤2%; grade 2 MI 2-20, Ki-67>2-20%; grade 3 MI >20, Ki-67 >20%. If there was a discrepancy between MI and Ki-67, grade was based on the higher score. Presence of necrosis excluded grade 1, regardless of MI or Ki-67 index. The resection specimens were graded according to the WHO 2010 classification system.
Results: The FNA and resection specimen grade were concordant in 25/31 (81%) cases. 25/31 (81%) of FNA cell blocks had greater than 500 cells present for evaluation. Three cases (9.7%) were upgraded from grade 1 to 2 based on a Ki-67 >2%; one of these cases had fewer than 500 cells on the cell block section. Three cases (9.7%) were downgraded from grade 2 to 1 based on a Ki-67 ≤2%; all three cases had greater than 500 cells on the cell block sections. The single neuroendocrine carcinoma in our study was scored as grade 3 on both the FNA and resection specimens; it was the only case with necrosis identifiable on FNA. In 30/31 FNA and all resection specimens, the Ki-67 proliferation index was higher than the mitotic index and determined tumor grade.
Conclusions: Pancreatic NET grade determined on FNA material showed good concordance (81%) with tumor grade on the resection specimen with no apparent discordant grade 1/2 vs grade 3 tumors, although the number of grade 3 tumors was limited. Grade was based primarily on the Ki-67 proliferation index, which was scored on a 500 cell count.

450 Cytotechnologist Assisted Adequacy Assessment of Image Guided Fine Needle Aspiration Specimens

Joshua Segal, Carol Anderson, Christina S Kong. Stanford Health Care, Stanford, CA.
Background: The volume of image-guided fine needle aspiration biopsy (FNAB) has markedly increased in recent years, and performance of on-site adequacy assessment has placed a significant burden on pathologist workload. In contrast, Pap volume has declined nationwide and resulted in a shrinking job market for screening cytotechnologists (CT). At our institution, we utilize CTs for on-site adequacy assessments. In this study, we assess the performance of CTs and the impact on workload.
Design: The CT log of time spent per case for on-site adequacy assessment of image-guided FNABs was evaluated for a six-month time period (February to August 2015) for amount of CT time per case and immediate interpretation. The on-site adequacy assessments were based on air-dried Diff-Quik stained slides and categorized as adequate, equivocal, or inadequate. CTs had access to an on-call cytopathologist as needed. The CT assessment was classified as discrepant if the initial assessment was "adequate" and final cytologic diagnosis was inconclusive, or if the assessment was "inadequate" or "equivocal" and the final cytologic diagnosis was malignant. Since not all nodules are malignant, final determination of inadequate specimens was based on identifying reports with diagnoses of "insufficient" or "limited sample".
Results: 821 FNABs with CT on-site adequacy assessments were documented in this six-month period. CTs spent a total of 491 hours (12.3 work weeks), average 36 minutes per case, performing adequacy assessments.

	ADEQUATE	INADEQUATE	EQUIVOCAL
CONCORDANT	99.5% (566/569)	71.3% (82/115)	72.1% (98/136)
DISCREPANT	0.5% (3/569)	28.7% (33/115)	27.9% (38/136)
TOTAL	69.3% (569/821)	14% (115/821)	16.6% (136/821)

Documentation was missing in 1 (0.1%) case. 55/821 (6.6%) FNABs ultimately yielded material that was insufficient for complete diagnosis. Of these, 52 (94.5%) were correctly assessed as inadequate (31/55; 56.4%) or equivocal (21/55; 38.2%) at the time of adequacy assessment.
Conclusions: Cytotechnologists are highly skilled morphologists who can be trained to accurately perform on-site adequacy assessment for image-guided FNAB. Rapid on-site assessment minimizes the rate of insufficient specimens and ensures that the material is appropriately prepared and triaged for ancillary diagnostic studies. CTs with sustainable roles in the hospital have the potential to save time and money for pathologists and clinicians, and streamline care for patients.

451 Accuracy of Intraoperative Imprint Cytology Performed Exclusively by Cytopathologists of Sentinel Lymph Nodes in Breast Cancer

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Background: There are several studies in the literature that explore the accuracy of intraoperative imprint cytology (IIC) of sentinel lymph nodes (SLN) in breast carcinoma. In our institution, these interpretations are performed exclusively by cytopathologists. We retrospectively analyzed the accuracy of our IIC compared to published data.

Design: A pathology database search of all cases from January 2013 to August 2015 was performed to identify axillary SLN with IIC from patients with a history of breast cancer. We recorded the correlation with final diagnosis, reason for any discrepancies, cytopathologist for each IIC, final diagnosis, and type of cancer.

Results: 226 axillary SLN were examined from 85 patients with histories of infiltrating duct carcinoma (IDC, 202 total nodes), microinvasive carcinoma (1), infiltrating lobular carcinoma (ILC, 8), duct carcinoma in situ (DCIS,10), or contralateral IDC but no disease in the ipsilateral breast (5). Discordance between the IIC and final diagnosis was noted for 12 SLN (5.0%). 3/12 discrepancies proved to be macrometastases of 8mm, 2.2mm and 2.05mm on histologic sections (N1a). On retrospective review, tumor was present on the IIC slide. In the 8mm focus, tumor was in a single cell pattern and the imprint was poor quality. In the other 2 cases, there were few tumor cells and they tended to aggregate along the edge of the smear where the lymphocytes were thicker. Of the other 9 discrepancies, 4 proved to be isolated tumor cells (ITC, N0) and 5 were micrometastases (N1mi) on histology. There were no false negatives among the ILC (4 true positives and 4 true negatives). On final histologic diagnosis, 26 of the 226 SLN were positive for tumor cells with 6 of these being ITC (N0); 7 micrometastases (N1mi), and 13 macrometastases (N1a). The sensitivities for N0, N1mi and N1a were 33% (2/6), 29% (2/7) and 77% (10/13) respectively, with overall sensitivity for N1 disease of 60% (12/20). Specificity was 100%. Published overall sensitivities for N1 disease range from 34% to 91%, with lower rates reported for micrometastasis (4% to 21%).

Conclusions: Although published reports do not consistently specify whether ICC was performed by a cytopathologist versus a non-cytopathologist, our overall sensitivities are in line with the admittedly broad range reported for all N1 disease. Our sensitivity for micrometastasis is slightly higher than reported sensitivities, suggesting that cytopathologists may be more attuned to the presence of a very small number of tumor cells in a lymphoid background.

452 RAS Mutations Are the Most Common Genetic Alteration in Indeterminate Thyroid Cytology -2 Years Institutional Experience

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Background: Widespread acceptance and implementation of molecular diagnostics in the management of thyroid nodules with indeterminate thyroid cytology is limited by the paucity of independent studies validating clinical sensitivity. We present 2 years of experience at our institution using a non-commercial molecular panel for triage of indeterminate thyroid cytology specimens.

Design: As part of routine clinical practice, thyroid FNAs with indeterminate cytologic diagnosis (atypia of undetermined significance, suspicious for follicular lesion, and suspicious for malignancy) were submitted to an outside academic institution for targeted mutation detection by PCR or by next generation sequencing. Review of the EMR was performed to correlate cytomorphologic and molecular results with the histology of surgically resected lesions.

Results: 1172 thyroid FNAs were performed over 27 months. 235 aspirates (21%) with an indeterminate diagnosis were submitted for molecular studies. Mutations were detected in 64 of 235 cases (27%). Mutations of RAS-family genes were most frequent (51%) with NRAS the most commonly mutated gene overall (30%). BRAF was the second most frequently mutated gene (24%). 26 of 33 RAS mutated cases had surgical follow-up and histologic-molecular correlation showed that 21 of 26 (81%) cases were neoplastic, while 5 were non-neoplastic (19%). Follicular adenoma was the most common neoplasm (39%) with RAS mutations followed by follicular carcinoma (15%), papillary thyroid carcinoma (15%) and follicular variant of papillary thyroid carcinoma (12%).

Conclusions: In patients with indeterminate thyroid cytology, RAS mutations are the most frequent genetic alteration. Follicular neoplasms including follicular adenoma, follicular carcinoma and follicular variant of papillary carcinoma, show the highest rate of harboring RAS mutations. Our results support recently published findings by other investigators also demonstrating that RAS mutations are the most common genetic alterations detected in indeterminate thyroid cytology.

453 Utility of Folate Receptor Alpha (FRA) Immunohistochemistry (IHC) in Cell Blocks (CB) of Breast Ductal Carcinoma, Müllerian Serous Carcinoma and Lung Adenocarcinoma

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Background: FRA overexpression by IHC has been shown to various degrees in histologic specimens from breast ductal carcinoma (50-86% of triple-negative {TNBC}), Müllerian serous carcinoma (~100%), lung adenocarcinoma (ADC) (~70%). In recent clinical trials, antifolate therapies have shown usefulness in FRA overexpressing malignancies. So far, FRA assessment in CB has not been studied. We assessed the feasibility for detecting FRA overexpression by IHC in CB from the aforementioned carcinomas.

Design: Our study included cases of breast ductal carcinoma, serous carcinoma of Müllerian origin and lung ADC. The CB were immunostained with FRA employing *mAb 26B.3.F2* (Biocare Medical, Concord, CA). FRA staining was scored qualitatively, by intensity, and staining area. A positive result was defined as >5% of the cells demonstrating membranous staining with ≥1+ intensity.

Results: The results are summarized in table 1.

Table1. FRA Immunostaining in Cytologic Specimens of Breast Ductal Carcinoma, Müllerian Serous Carcinoma, and Lung ADC.

	FRA IHC ***mAb 26B.3.F2*** (Biocare Medical, Concord, CA)
Breast Ductal Carcinoma:	
Triple Negative *(n=20)*	5 (20%)
ER/PR Positive *(n=5)*	0
Her2/neu only Positive *(n=5)*	0
MÃ¼llerian Serous Carcinoma *(n=29)*	27 (93%)
Lung Adenocarcinoma, NOS *(n=22)*	20 (91%)

The FRA immunohistochemical staining characteristics are shown in figure 1.

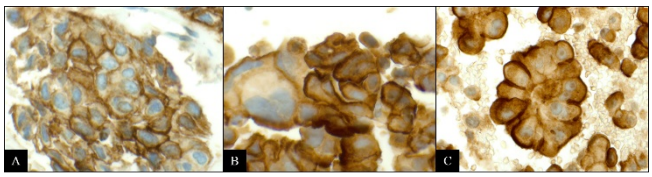

Figure1. Positive FRA IHC in Cytology Cell Blocks (x600): (A) Triple negative breast ductal carcinoma, (B) Serous Carcinoma from Müllerian origin, and (C) Lung ADC.

Conclusions: FRA expression can be detected with a higher degree of confidence in Müllerian serous carcinoma (93%) and lung ADC (91%) in CB, and to a lesser degree in TNBC. Our data also shows that FRA expression by IHC was more frequently associated with TNBC (20%) when compared with ER/PR positive or Her2-neu positive breast cancers. FRA overexpression detected by IHC in CB is highly concordant with the published results of surgical specimens from Müllerian and lung adenocarcinoma and less so from TNBC. Hence, IHC FRA analysis of CB may be utilized for treatment decisions with antifolate therapy with a high degree of confidence in these malignancies.

454 Human Papillomavirus (HPV) Genotypes and Anal Squamous Intraepithelial Neoplasia (AIN) in HIV Positive Women

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Background: Anal cytology screening and HPV testing among high risk populations have been well established. However, the most studies have been focused on men. This study is to investigate the association of HPV genotyping and cytologyic diagnosis with histologic diagnosis in HIV positive women.

Design: Between 8/2012 and 12/2014, 315 patients had Pap smear and HPV testing. HPV genotyping was performed by PCR (cobas® HPV Test, Roche) and positive HPV results were reported as 16, 18 and Other (OhrHPV)-31/33/35/39/45/51/52/56/58/59/66/68).

Results: In 315 patients, the cytologic diagnoses were Negative 94 (29.84%), ASCUS 143 (45.4%), ASC-H 11(3.49%), LSIL 55 (17.46%) and HSIL 12 (3.80%). HPV infection was found in 220 patients (69.84%). By HPV genotyping, patients infected by HPV 16, 18 and OhrHPV are 29.09% (64/220), 27.72% (62/220) and 93.18% (205/220). The HPV infection was different among cytology classifications (Table 1). Of 125 patients with anal biopsy, the histologic diagnoses were benign 25(20%), AIN1 50 (40%) and AIN2-3 50 (40%). AIN was found almost in HPV positive patients: 90% (45/50) in AIN1 and 96% (48/50) in AIN2-3. In HPV positive patients, AIN were more commonly seen in cytologically abnormal patients (Table 2). Although HPV genotypes were similar regardless of biopsy results, co-infection of HPV (16 or 18 with OhrHPV) was found to be significantly different in patients with AIN2-3, 50% in biopsy negative patients, 42.2.% in AIN1 patients and 68.75% in AIN2-3 patients. 238 patients had concurrent anal and cervical HPV testing. The HPV positive rate were 68.49% in anal vs. 34.87% in cervical.

Cytology Dx (case#)	HPV positive	HPV negative	% of positive
Negative (94)	46	48	48.94
ASCUS (143)	99	44	69.23
LSIL (55)	52	3	94.5
ASC-H (11)	11	0	100
HSIL (12)	12	0	100
Total	220	95	

Cytology Dx	Biopsy Dx	%	Biopsy Dx		Biopsy Dx	
	Benign		AIN1	%	AIN2-3	%
Negative	6	40	6	40	3	20
ASCUS	9	18.75	21	43.75	18	37.5
LSIL	2	7.14	17	60.71	9	32.14
ASC-H	1	10	1	10	8	80
HSIL	0	0	0	0	10	100
total	18		45		48	

Conclusions: 1. OhrHPVs are the most common HPV genotypes in HIV positive womwn. 2. AIN is more frequently found in HPV positive patients with abnormal cytology. 3. Co-infection of HPV in AIN2-3 is higher. 4. The incidence of HPV infection in anal is significant higher than that in cervical

455 Glandular Findings in Cervical Cytopathology Liquid Based Preparations in Cases of Endometritis: A Series of 250 Patients

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Background: Chronic endometritis has been reported to cause glandular abnormalities in a Pap smear. However, the rate and types of these glandular abnormalities have not been studied in a large series. We investigated glandular findings on cervical cytopathology liquid based preparations, both ThinPrep and SurePath, from patients with endometritis. To the best of our knowledge, no study has examined such a large series of histologically proven endometritis or compared the rates of glandular findings on the two different types of liquid based preparations, ThinPrep and SurePath.

Design: Surgical pathology records from the University of New Mexico were reviewed from July 2009 through July 2015 and cases of endometritis, diagnosed histologically, were identified. The study set consisted of 250 cases with a corresponding Pap monolayer slide received within one year of the surgical case. Patient age and time lag between the surgical case and Pap were recorded. All 250 Paps were reviewed and type of liquid based preparation (SurePath or ThinPrep); presence of endocervical cells; and presence of benign endometrial cells (BECs), atypical endometrial cells (AECs), and atypical glandular cells not otherwise specified (AGCs) were recorded.

Results: Of the 250 Paps examined, 166 (66%) were SurePath and 84 (34%) were ThinPrep. Of the 250 cases, 20.3% had BECs, AECs, and/or AGCs. SurePath identified significantly more cases with BECs, 31 (18.7%) vs. 9 (10.7%), and AECs, 8 (4.8%) vs. 0 (p-value = 0.012) when compared to ThinPrep. Of note, when BECs, AECs, and AGCs were grouped together, the statistical significance was lost (p = .076), likely owing to the fact that endometritis is a relatively uncommon entity and this is reflected in our sample sizes. No statistically significant difference in the rate of identification of BECs, AECs, or AGCs was identified based on the patient's age (comparing women aged 45 or older versus those under 45) or time lag between cervical Pap and surgical case.

Patient Age	<45: 156 (62%)	>=45: 94 (38%)
Liquid Based Prep Type	Sure Path: 166 (66%)	ThinPrep: 84 (34%)
Endometrial Cells	None: 200 (80%)	BEC, AEC, AGC: 50 (20%)

Conclusions: A relatively high rate of glandular findings prevails in patients with endometritis (~20%). SurePath cervical cytopathology preparations are statistically more sensitive than ThinPrep preparations at detecting the presence of benign endometrial cells and atypical endometrial cells in patients with endometritis confirmed histopathologically within one year of cervical cytopathology.

456 Label-Free Enumeration, Collection and Downstream Cytological and Cytogenetic Analysis of Circulating Tumor Cells

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Background: Circulating tumor cells (CTCs) have a great potential as indicators of metastatic disease that may help physicians improve cancer treatment and patient outcomes. The current FDA-approved method of isolation and characterization relies on antibodies targeted towards cell surface markers, but heterogeneous marker expression as well as the complexity of isolation and analysis systems highlights the need for alternative methods.

Design: In this work, we use a recently introduced microfluidic Vortex trapping device that can selectively isolate and concentrate large cells from blood independently of cell surface expression. This system was adapted to interface with three protein-marker-free analysis techniques: i) an in-flow automated image processing system to enumerate cells released, ii) cytological analysis using Papanicolaou staining and iii) fluorescence in situ hybridization targeting the ALK translocation. In-flow counting enables a rapid assessment of the potential levels of CTCs in a blood sample within minutes to determine whether standard downstream assays that are more time consuming and costly would be warranted. Blood samples were drawn from patients, diagnosed with metastatic non-small cell lung cancer (n=10, NSCLC) and metastaticbreast cancer (n=6), as well as 5 healthy volunteers.

Results: Using this device, CTCs isolated from 10 NSCLC and 6 breast cancer patient samples were collected and stained with conventional Papanicolaou stain (Pap stain). Malignant cells were detected in 9 of the 10 lung samples and 6 of the 6 breast samples, while no malignant cells were detected in 5 healthy donors. As a prove of principal, cytogenetic analysis was performed succesfully in one anaplastic lymphoma receptor tyrosine kinase (ALK)+ and one ALK- sample.

Conclusions: Though further validation is needed, the preliminary study demonstrated that the Vortex system shows promise as a rapid sample preparation approach for blood samples to yield diagnostic cells for downstream cytopathology, expanding the range of samples that can be analyzed in the cytopathology and cytogenetics labs, and ultimately aiding in cancer diagnosis, treatment monitoring, and personalized therapy selection.

457 Prevalence of HPV 16/18 Genotypes and Histological Follow-Up Results in Women with Negative Pap Cytology/Positive Cervista HPV HR Assay

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Background: Cytology-negative/HPV-positive cases were found in 4% of the cotest results. The outcomes of cytology-negative/Cervista HPV HR-positive have not been evaluated in routine clinical practice.

Design: A computer-based search of databases was conducted to identify cases with negative Pap test/positive Cervista HPV HR testing from 6/2013 to 5/2015. And cases with Cervista HPV16/18 genotyping were also analyzed.

Results: Of 1,907 Pap-negative/Cervista HPV HR positive cases, 1826 (95.8%) had Cervista HPV 16/18 genotyping results. 313 patients (17.1%) tested positive for either HPV 16 or 18 (17.1%), with the majority positive for HPV16 (15.7%) compared to HPV18 (1.3%) and 16/18 (0.6%). Non-16/18 HR HPV types accounted for 82.8%. Age and follow-up results are listed in table 1. Histological follow-up results were found in 179 of 313 women (57.2%) with positive HPV16/18 over an average of 2.4 months (0.3-15 m) and in 190 of 1513 (12.6%) women with non-16/18 HR HPV types over an average of 5.6 months (0.3-18 m). The average ages are the same between the women with HPV16/18 group and women with non-16/18 group. CIN2/3 detection rate in HPV16/18 group appears higher (5.0%, 9/179) than in non-16/18 HR HPV group (2.1%, 4/190) in a short follow-up period. The average follow-up period for cases with CIN2/3 detection was 1.4 months (0.7-2 m) in HPV16/18 group and 9 months (1-17 m) in non-16/18 group.

HR HPV types	Total case#	Average age (range)	Biopsy# (%)	CIN2/3# (%)	CIN1# (%)	Negative# (%)	Average Biopsy Period, M (range)
16	286	47.1 (25-82)	158 (55.2)	8 (5.1)	46 (29.1)	104 (65.8)	2.4 (0.3-15)
18	24	44.5 (30-75)	18 (75)	1 (5.6)	7 (38.9)	10 (55.6)	2.2 (0.7-13)
Both	3	38 (30-51)	3 (100)	0	0	3 (100)	2.8 (1-6)
16 and/or 18	313	46.8 (25-82)	179 (57.2)	9 (5.0)	53 (29.6)	117 (65.4)	2.4 (0.3-15)
Non-16/18	1513	46.7 (21-79)	190 (12.6)	4 (2.1)	55 (28.9)	131 (69.0)	5.6 (0.3-18)
Total	1826	46.7 (21-82)	369 (20.2)	13 (3.5)	108 (29.3)	248 (67.2)	4.1 (0.3-18)

Conclusions: The rate of positive HPV16/18 in women with negative Pap/positive Cervista HPV HR assay is significantly higher than that of the women with negative Pap/positive HC2 HPV assay in our previous study (17.1% vs 12.3%, p<0.05). Short-term follow-up results demonstrate the rate of CIN2/3 detection in women with negative Pap/HPV16/18 is higher than in women with negative Pap/non-16/18 HR HPV types, supporting ASCCP guideline: routine HPV16/18 triage test for women with negative Pap test/positive HR HPV testing.

458 The Cytologic Diagnosis of Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma: A Prospective Analysis

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Background: At the 2015 USCAP Companion Meeting, the Endocrine Pathology Society announced a proposal to change the terminology for non-invasive follicular variant of papillary thyroid carcinoma (NFVPTC) to reflect the indolent clinical behavior of this tumor and encourage conservative management (i.e., lobectomy only). The Bethesda System for Reporting Thyroid Cytopathology does not specifically account for this class of tumors, but identifying them at the time of fine needle aspiration (FNA) would be clinically important so that patients might be treated with lobectomy instead of total thyroidectomy. The aim of this study was to determine if NFVPTCs can be distinguished from classical PTCs (cPTCs) at the time of FNA.

Design: Beginning in June 2015, all FNAs with a cytologic diagnosis of "malignant" or "suspicious for PTC" (i.e., diagnoses that are likely to trigger total thyroidectomy) were prospectively scored by the reviewing cytopathologist for features associated with NFVPTC (microfollicular architecture, absence of papillae, and absence/scarcity of nuclear pseudoinclusions). Based on these features, each case was categorized as NFVPTC, cPTC, or indeterminate. The FNA results were correlated with histologic diagnoses of resection specimens.

Results: 20 FNAs with resection correlates were available for preliminary analysis (additional data being accrued). The histopathologic diagnoses included 13 cPTC, 6 FVPTC (including 3 NFVPTC and 3 FVPTC with invasion), and 1 poorly differentiated thyroid carcinoma. Of the 13 cPTCs, 12 (92%) cases were correctly categorized at the time of FNA, with only 1 (8%) case mistaken for NFVPTC (a cPTC with a predominantly follicular architecture). Of the 6 cases of FVPTC, cytologists categorized 3 (50%) as NFVPTC (2 were NFVPTC and 1 was an FVPTC with invasion), 2 (33%) as cPTC, and 1 (17%) as indeterminate. The presence of pseudoinclusions (p = 0.046) and papillae (0.057) were predictors of cPTC while microfollicular predominance (p = 0.262) was not as predictive of subtype.

Conclusions: The preliminary results indicate that NFVPTC frequently can be differentiated from cPTC by thyroid cytology. Additionally, these results suggest that cPTCs with a predominantly follicular architecture and FVPTCs with invasion are potential confounders of cytology-based subtype prediction.

459 Development of a Diagnostic Model Using Key Cytologic Features in Washed Urine Cytology

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Background: Various cytologic features have been described in washed urine cytology for differential diagnosis between reactive urothelial atypia and urothelial tumors. However, diagnostic significance of the cytologic features has not been defined yet. We aimed to define key cytologic features of washed urine cytology and to generate diagnostic model for the differential diagnosis.

Design: 369 cases with biopsy-proven urothelial diseases and washed urine cytology at our institution between 2010 and 2013 were selected and fourteen features were evaluated on washed urine cytology slides. To generate a diagnostic model, a developmental dataset (369 cases, 2010-2013) was developed by one pathologist. In addition, an external validation dataset (105 cases, 2011) was constructed by another pathologist. Statistically, backwards step-down procedure was done to select diagnostic cytologic features and to build diagnostic modeling.

Results: Seven cytologic features are important for differential diagnosis between reactive urothelial atypia and urothelial tumors in washed urine cytology. They are tumor diathesis, ragged edges, diffuse anisonucleosis, eccentric nuclei, altered chromatin, coal-black nuclei and apoptotic bodies. A diagnostic model is generated using the seven cytologic features and its accuracy is 82.9%. On external validation, the diagnostic accuracy is 78.1% with 100% of specificity, 82.6% detection rate of high grade urothelial carcinoma, and 11.8% detection rate of low grade urothelial tumors.

Conclusions: Seven cytologic features are important and selected for diagnostic model building to differentiate reactive atypia from urothelial tumors in washed urine cytology with good diagnostic accuracy. However, a further study is required to increase diagnostic accuracy, especially of low grade urothelial carcinoma.

460 Molecular Testing (MT) on Endobronchial Ultrasound (EBUS) Fine Needle Aspirates (FNA): Significance of Appropriate Specimen Triage (ST) at Start of Procedure

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Background: EBUS-guided FNA has become the standard of care in diagnosing and staging patients with lung cancer (LC). The CAP, IASLC and AMP recommend *EGFR* mutation and *ALK* rearrangement testing for all patients with advanced stage lung adenocarcinoma and an even broader panel is tested for clinical trials. EBUS samples are small and the success of MT is variable. At our institution, for EBUS FNA we have a protocol for managing specimens and provide rapid on-site evaluation (ROSE) for all cases. Several factors during ST (e.g. operator, procedural skill, lab technique, etc.) can contribute to the outcome of MT. The aim of this study is to assess the impact of ST on MT.

Design: A computerized retrospective search of consecutive EBUS FNAs performed by 2 pulmonologists (PMs) over a 32-month period was performed. All adenocarcinomas, non-small cell carcinomas, adenosquamous cell carcinomas and poorly differentiated carcinomas were selected. The cases were divided into two groups: (A) ST performed by a cytopathologist (CP) at start of the procedure and (B) ST performed by a non-CP or CP after start of procedure. ST is defined as utilization of the smallest necessary amount of tissue for ROSE and allocation of the remainder for cell block and MT. All specimens underwent reflex MT in our molecular pathology lab (MPL), with the typical panel consisting of *EGFR*, *KRAS* and *ALK*, but this has varied/expanded overtime with test availability. The MT outcomes of the two groups were analyzed using "N-1" chi-squared and Fisher exact tests.

Results: A total of 104 patients (ages 43-93, 60% female, 40% male) were identified, including 24 from Group A and 80 from Group B. One (4%) and 21 (26%) of cases had insufficient sample for MT from Groups A and B, respectively. The data was statistically significant using "N-1" chi-squared and Fisher exact tests and yielded p-values of 0.021 and 0.022, respectively.

Conclusions: We found that the most significant variable affecting the outcome of MT was ST, which is the first in a series of multiple downstream specimen handling steps. Confounding by operator, procedural skill, and lab technique is unlikely as our retrospective cohort used the same 2 PMs, CPs, and MPL. This significant difference could be due to more efficient ROSE with greater emphasis on MT when triaged by a CP at start of the procedure. With the continual identification of genes implicated in LC and potential therapies, ST will play an even more significant role.

461 Performance Characteristics of Body Fluid Cytology (BFC): Analysis of 344,380 Responses from the College of American Pathologists (CAP) Non-Gynecologic Cytopathology Education (NGC) Program

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Background: BFC is an important diagnostic test for the assessment of a variety of conditions. Distinguishing benign from malignant changes and identifying specific malignancies in this setting can present challenges. This study was undertaken to identify performance characteristics of BFC in the CAP NGC program.

Design: 344,380 responses based on 5102 slides evaluated in years 2002-2013 were analyzed for concordance to the general category (GC) and reference diagnosis (RD). Two nonlinear mixed models were used to analyze the concordance rates (CR).

Results: The GC (benign vs. malignant) CR was 95.2%. GC type, participant type and slide preparation were significantly associated with concordance to the GC (*P*-value <.001). Malignant cases performed better than benign ones (95.8% vs. 84.2%); cytotechnologists (CT) had better GC CR than pathologists (MD) (95.6% vs. 94.8%); ThinPrep preparations (TPP) had the highest GC concordance (96.4%), and SurePath slides had 99.7% concordance in pericardial fluids (PCF); however nearly all of these PCF challenges were adenocarcinomas. Fluid type, specific RD, participant type, and slide preparation were significantly associated with concordance to the RD (*P*-value <.001). Adenocarcinoma showed one of the highest GC (96.0%) and specific RD (76.9%) performance, and was the most common challenge in all fluid sites. MD performed better than CT for concordance to the RD (71.6% vs 69.7%). PCF had the lowest RD concordance (65.5%). Modified Giemsa (MG) preparations performed best for lymphoma/ hematopoietic lesions (L/H): 88.7% of pleural RD were concordant. PCF showed the least concordance for a benign/reactive diagnosis. Small cell (SmCC) carcinoma showed the highest GC CR (98.4%) and higher CR with the RD in pleural (83.3%) than in peritoneal fluids (PTF) (45.1%).

Conclusions: This review illustrates the difficulties in achieving accurate diagnosis in BFC samples, particularly in PCF which showed the least concordance to the RD. The lower benign category concordance may be due to "look-alike entities", the negative slides in the program presenting a greater challenge than routine negative slides, or related to the "test" setting where participants may be reluctant to call a sample negative. The results also highlight the challenges of accurately differentiating SmCC from L/H and lymphocytic inflammation in PTF and the value of MG preparations for L/H.

462 The Pap Test Reporting Rates for Conventional Pap Tests and Liquid-Based Cervical Cytology from the Largest Academic Women's Hospital in China: Analysis of 1248785 Papanicolaou Test Reports

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Background: Data on cervical Pap test results in literatures are very limited in China. Between 2009 and 2014, Pap testing changed significantly in China.

Design: A retrospective database search documented all Pap test reports between 2009 and 2014 by both specimen type, LBC and conventional Pap smears (CPS), and report category. Totally 1,224,785 Pap were analyzed.

Results: LBC gradually largely replaced the CPS, which declined from over 65% of Pap tests in 2010 to 6.4% in 2014. Class I-II accounts for 99.2% Pap tests, totally 0.34% for class III-V. Overall, the negative rate is higher in PCS than TBS reporting. The laboratory abnormal reporting rate increased for all TBS categories as LBC replaced the CPS and after technician reviews were replaced by pathologist reviews. TBS categories from this large laboratory were generally within benchmark ranges reported by CAP, except for very low rate of unsatisfactory and atypical glandular cells, and relatively low ASC-US reporting rate. (table 1)

Table 1 The TBS rate of each preparations.

Categories	LBC			CPT	Total	Age
	ThinPrep	SurePath	Total			
ASC-US (%)	5753 (2.2)	7404 (2.3)	13157 (2.26)	770 (0.5)	13927 (1.9)	38.5 (15,89)
ASC-H (%)	551 (0.2)	711 (0.2)	1262 (0.22)	19 (0.0)	1281 (0.2)	43.0 (19,90)
LSIL (%)	3373 (1.3)	5094 (1.6)	8467 (1.46)	239 (0.2)	8706 (1.2)	36.8 (15,85)
HSIL (%)	1233 (0.5)	1562 (0.5)	2795 (0.49)	93 (0.1)	2888 (0.4)	41.7 (19,86)
SCC (%)	282 (0.11)	288 (0.09)	570 (0.10)	14 (0.01)	584 (0.08)	50.3 (18,90)
AGC (%)	108 (0.04)	176 (0.05)	284 (0.05)	13 (0.01)	297 (0.04)	42.4 (19,81)
AIS/AD (%)	54 (0.02)	56 (0.02)	110 (0.02)	8 (0.01)	118 (0.02)	51.1 (24,80)
NILM (%)	248698 (95.5)	305697 (95.2)	554395 (95.35)	149225 (97.8)	703620 (95.9)	37.3 (15,97)
Unsat	335 (0.13)	50 (0.02)	385 (0.07)	2168 (1.42)	2553 (0.35)	42.6 (25,88)
ASC:SIL	1.4	1.2	1.28	2.4	1.3	
Total	260387	321038	581425	152549	733974	37.4 (15-97)

Conclusions: Cervical screening test results in this study impact of both introduction of standardized TBS reporting and increased use of liquid-based cytology. Reporting of all TBS abnormalities increased using LBC.

463 Endometrial Cells on Pap Testing: Impact of Revisions to the Bethesda System for Reporting Cervical Cytology

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Background: Benign endometrial cells on Pap tests may herald the discovery of endometrial abnormalities. Recently, in the 2014 3rd edition of The Bethesda Reporting System, the age of reporting endometrial cells was raised from 40 to 45. Our study aims to determine the impact of this change on our patient cohort, especially regarding the potential underdetection of significant endometrial lesions.

Design: Our database was searched for benign endometrial cells found on Pap tests between January 2005 and May 2015. Data was stratified betwen premenopausal women aged 40-44, premenopausal women aged 45 years and older, and postmenopausal women. Histologic follow-up was obtained, and chi-square statistical analysis was performed.

Results: During our 10-year study period, 1,036,576 Paps were examined. Of these, 4438 patients (0.4%) over age of 40 had benign endometrial cells on their Pap tests. Between the 3 groups, postmenopausal women demonstrated significantly more (7.1%) histologic abnormalities, compared to 1.0% and 0.4% of premenopausal women aged >45 years and premenopausal women aged 40-44, respectively (P<0.001).

The rate of abnormalities found in premenopausal women between the ages of 40-44 was significantly lower than premenopausal women over age 45 (P = 0.04) and postmenopausal women (P<0.0001). In the younger premenopausal group, atypical hyperplasia and cancer were diagnosed in 4 (2%) patients, aged 40-41, with abnormal bleeding symptoms in 2(50%). There were only 2 low grade endometrioid adenocarcinomas in this younger group.

Among the premenopausal women aged 45 and older, 13(5%) patients demonstrated atypical hyperplasia or cancer, aged 45-55 (average 49), with abnormal bleeding symptoms in 7(54%). There were 9 cancers in this group, including 4 high grade malignancies.

Conclusions: Our study demonstrated that only a few asymptomatic low grade cancers will be missed, if reporting of benign endometrial cells in the premenopausal group aged 40-44 is discontinued. In contrast, high grade cancers were detected in our premenopausal patients over 45, supporting the recent Bethesda guideline revisions.

	Premenopausal 40-44	Premenopausal >45	Postmenopausal	Total
# of Cases	1912	2186	340	4438
Average Age (Range)	42(40-44)	48(45-59)	58(45-89)	46(40-89)
Biopsies #(%)	202(11%)	268(12%)	137(40%)	608(14%)
Abnormal Bleeding #(%)	96(5.0%)	94(4.3%)	61(18%)	251(5.7%)
Abnormal Histology #(%)	8(0.4%)	21(1.0%)	24(7.1%)	53(1.2%)
Cancers #(%)	2(0.1%)	9(0.4%)	12(3.8%)	24(0.5%)

464 HPV Testing of Squamous Cell Carcinomas in Non-Gynecological Cytology: Concordance of p16 Immunostaining and In Situ Hybridization for HPV

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Background: The discovery of Human Papillomavirus (HPV)-related squamous cell carcinomas (SqCC) has allowed HPV testing to determine the origin of metastases from tumors of the uterine cervix, anus, or head and neck (H&N), while also providing prognostic data and eligibility for clinical trials. The aim of this study was to determine the concordance of HPV testing by in-situ hybridization (ISH) and p16 immunohistochemical staining (IHC) in non-gynecological (non-GYN) cytology specimens of SqCC.

Design: Non-GYN cytology specimens between January 2014 and May 2015 with a final diagnosis of SqCC with HPV testing performed were selected. HPV testing included HPV ISH (ENZ-32884 PathoGene; positive was definitive punctate staining in tumor) and/or p16 IHC (E6H4, Ventana; positive was strong nuclear and cytoplasmic staining in >70% of tumor) analyzed on cell block material and correlated with follow up.

Results: A total of 46 cytology cases (1 exfoliative, 45 aspirations) were obtained from 46 patients (mean age 62 years, 67% male), with the majority of specimens from lymph nodes (74%). HPV ISH testing and p16 IHC was performed on 45 (98%) and 41 (89%) specimens, respectively. HPV and p16 were informative in 33 (72%) cases and concordant in 79%.

Table 1

	Cytology p16 IHC +	Cytology p16 IHC -
Cytology HPV ISH +	19 (58%)	1
Cytology HPV ISH -	6	7

They were non-informative (15% HPV ISH, 5% p16 IHC) due to equivocal staining or stain failure. The site of origin was confirmed to be HPV-related in 25 cases with follow-up (1 (4%) anal, 1 (4%) cervix, 7 (28%) tonsillar, 10 (40%) base of tongue and 6 (24%) other H&N sites), of which 16 (64%) were positive for HPV ISH and/or p16 IHC. The sensitivity, specificity, positive predictive value and negative predictive value for HPV testing by cytology, using available histology and clinical follow-up, is 76%, 100%, 100%, and 44% respectively.

Table 2

	HPV related SqCC on Follow-up	Non-HPV related SqCC on Follow-up
Cytology HPV testing +	16 (64%)	0 (0%)
Cytology HPV testing -	5 (20%)	4 (16%)

Conclusions: In summary, HPV ISH was performed more often than p16 IHC, and was concordant with p16 results in 79%, but had more indeterminate results than with p16 IHC in squamous cell carcinomas. Confirmation of an HPV-related SqCC was possible in 64% of non-GYN cytology cases with HPV testing and site-specific follow up. Overall, the HPV testing proved to be highly specific but only 76% sensitive.

465 EGFR Mutation Detection on Non Small Cell Lung Cancer Routine Cytological Samples by Digital PCR

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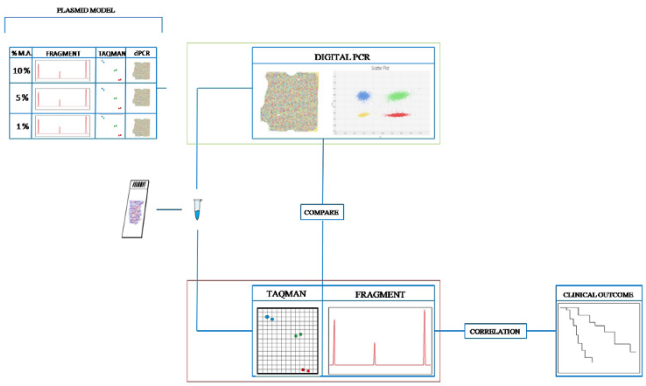
Background: Highly sensitive genotyping techniques are useful to detect epidermal growth factor receptor (EGFR) mutations on lung cancer cytological samples, when these feature only few neoplastic cells. This study aimed to validate digital polymerase chain reaction (dPCR) methodology on cytological material.

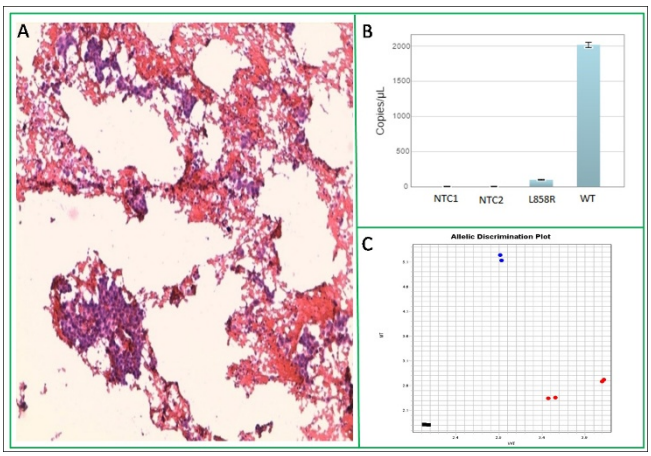
Design:



On the left, dPCR analytical sensitivity assessment is schematically reported. Assay sensitivity was assessed on a plasmid model (15%, 10% and 1% mutant allele) and compared to that shown by fragment length and TaqMan assays.

On the right, cytological samples (n= 30), previously tested by fragment length and TaqMan assays and with available clinical data, harboring exon 19 deletions (n= 8), L858R mutations (n= 2) and wild type DNA (n= 20), were retrospectively selected and retested by dPCR

Results: Results showed high concordance (96,6%). However, dPCR detected an additional L858R mutation that had been missed by TaqMan assay on a paucicellular smear. This mutation was confirmed by cloning PCR products and sequencing, as show in **Figure 2.**



Digital PCR detects L858R on DNA extracted from a paucicellular smear. The DNA extracted from a Papanicolaou stained smear (A), containing <10% of neoplastic cells undergo dPCR, As it is shown in (B), the dPCR technique detected in 98 copy/µl the presence of L858R mutation in a background of 2000 copy/µl wild type DNA. No template control (NCT) 1 and 2 did not show amplification signal. Note that the TaqMan assay (C) failed to detect the mutation.

Conclusions: Our data showed that dPCR may reliably expand the range of methods available in a centralized laboratory enabling the possibility to test even paucicellular smears.

466 On-Site Adequacy Evaluation Does Not Affect the Rate of Nondiagnostic Thyroid Fine Needle Aspiration

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Background: The utility of on-site adequacy evaluation (OSAE) for thyroid FNA is controversial. At our hospital, all thyroid FNAs are performed under ultrasound guidance. Most thyroid FNAs are collected in CytoLyt without OSAE, with multiple passes from each nodule processed in a single ThinPrep slide. In a minority of FNAs performed at select thyroid nodule clinics, cytopathology fellows evaluate air-dried direct smears for OSAE, with needle rinsings concurrently collected in CytoLyt for ThinPrep processing. We retrospectively evaluated whether OSAE affects the nondiagnostic (ND) rate of thyroid FNAs in our institution.

Design: Cytology records were searched for thyroid FNAs performed with and without OSAE between 8/2013 and 7/2015. The final adequacy determination of each case was recorded ("ND" or "diagnostic"). For cases with OSAE, adequacy results were recorded ("inadequate" or "adequate"). The timing of the FNA (1st-time versus repeat) and ultrasound evidence of cystic changes were noted.

Results: OSAE was provided for 281 (13.9%) of the 2016 thyroid FNAs performed in the study period. The ND rates for FNAs with and without OSAE were 15.7% and 15.2%, respectively. Of the cases with OSAE, 141 (50.2%) were inadequate at the time of OSAE; 100 of these were ultimately diagnostic because of sufficient cellularity in the corresponding ThinPrep slide. 6 cases were reported as adequate at the time of OSAE but downgraded to ND on final evaluation. For nodules that required reaspiration on separate dates (for indeterminate or non-diagnostic FNAs), OSAE made no significant difference in the final ND rate at the time of 1st or 2nd FNA; for nodules undergoing FNA for a 3rd time, OSAE was associated with a decrease in the ND rate (Table 1). OSAE did not make a significant difference in the ND rate among clinically cystic nodules (Table 1).

Conclusions: OSAE did not impact the final specimen adequacy rate except when the FNA was repeated for the 3rd time on the same nodule, albeit at significant expense to the cytology lab. While adequacy "upgrades" from the OSAE to the final diagnosis were common, "downgrades" were rare.

	Without onsite evaluation			With onsite evaluation		
	Total	ND	ND rate	Total	ND	ND rate
Total	1735	264	15.2%	281	44	15.7%
- Nodules with only 1 FNA	1272	136	10.7%	159	15	9.4%
- Nodules with multiple FNAs						
- 1st FNA	263	84	31.9%	27	8	29.6%
- 2nd FNA	176	36	20.5%	86	19	22.1%
- 3rd FNA	21	8	38.1%	9	2	22.2%
- 4th FNA	3	0	0%	0	0	-
- Clinically cystic						
- Yes	156	47	30.1%	24	8	33.3%
- No	1579	217	13.7%	257	36	14.0%

467 The Clinical Impact of Biliary Tract FISH Analysis

Nilam Virani, Madelyn Lew, Bryan Betz, Michael H Roh, Xin Jing, Amer Heider, Robertson Davenport, Judy C Pang. University of Michigan, Ann Arbor, MI.

Background: Bile duct brushings have a sensitivity of 10-72% and specificity approaching 100% for detecting malignancy. Given the limitations of cytology, indeterminate diagnoses are not infrequent, especially in patients with primary sclerosing cholangitis (PSC) where reactive changes can mimic carcinoma. As such, adjunct FISH testing using the UroVysion™ probe set has been advocated to improve the sensitivity of detecting malignancies over routine cytology alone. At our institution, a definitive diagnosis of malignancy is typically needed prior to initiation of therapy, especially if non-surgical, resulting in additional procedures for tissue diagnosis when cytology is indeterminate. We sought to investigate the impact of FISH on the clinical management of our patients.

Design: From January 2014 to August 2015, since the validation of UroVysion™ FISH for biliary tract malignancy at our lab, 63 bile duct brushings have been tested either at the request of the clinician or as reflex testing in patients with an indeterminate diagnosis on the concurrent cytology specimen. Patient outcomes were recorded.

Results: All negative cytology had negative FISH. The PSC cohort had a lower rate of FISH positivity than the non-PSC cohort for both atypical (6% vs 14%) and suspicious cytology (0 vs 40%). Except for one patient without follow up, all patients with positive FISH and 9 patients with negative FISH had clinical or tissue diagnosis of malignancy.

PSC					
	Cytology			**Malignant follow up**	
FISH	**Negative (n=4)**	**Atypical (n=18)**	**Suspicious (n=2)**	**Clinical**	**Tissue**
Positive	0	1	0	1	0
Negative	4	17	2	0	1
Non-PSC					
	Cytology			**Malignant follow up**	
FISH	**Negative (n=6)**	**Atypical (n=28)**	**Suspicious (n=5)**	**Clinical**	**Tissue**
Positive	0	4	2	1	4
Negative	5	23	3	2	7
Equivocal	0	1	0	0	0
Insufficient	1	0	0	0	0

Most patients had additional biopsy/cytology to confirm malignancy prior to treatment.

		Additional biopsy/cytology	
Cytology	**FISH**	**Yes**	**No**
Atypical	Positive	3	0
Atypical	Negative	4	1
Suspicious	Positive	1	1
Suspicious	Negative	2	1
Negative	Negative	1	0

Conclusions: FISH is a useful adjunct to cytology in the evaluation of bile duct brushings as a positive FISH correlates highly with malignancy. However, a negative FISH does not exclude a malignancy. This preliminary data suggests that definitive tissue diagnosis is still preferred prior to treatment, regardless of the FISH results.

468 Secondary Malignancies in Salivary Glands: A Multi-Institutional FNA Cohort from Trans-Atlantic Academic Institutions

He Wang, Raza S Hoda, William C Faquin, Esther Rossi, Nihar Hotchandani, Tianlin Sun, Marc Pusztaszeri, Tommaso Bizzarro, Massimo Bongiovanni, Viren Patel, Nirag Jhala, Yun Gong. Temple University Hospital, Philadelphia, PA; Massachusetts General Hospital, Boston, MA; Catholic University of Rome, Rome, Italy; MD Anderson Cancer Center, Houston, TX; Geneva University Hospital, Geneva, Switzerland; University of Lausanne, Lausanne, Switzerland.

Background: Secondary malignancies of the salivary glands (SGs), either metastatic from a known primary site or from direct extension of a surrounding neoplasm, are among the most common malignancies affecting the SG. Fine needle aspiration (FNA) of secondary SG neoplasms can present diagnostic challenges. Our study presents the largest such FNA series of secondary SG tumors.

Design: A search of pathology databases of six academic institutions (3 in Europe, 3 in USA) identified 189 FNA cases of secondary SG tumors. All initial diagnoses were rendered on SG FNA. The FNA diagnoses were correlated with the corresponding SG biopsies/resections when available.

Results: Secondary SG malignancies were more common in men (men:women =2.4:1), with an average age at diagnosis of 68.9 years (range: 2 – 92 years). Metastatic squamous cell carcinoma (SCC, n=79) and melanoma (n=61) constituted the majority of cases. Other skin-derived carcinomas included Merkel cell carcinoma (n=3) and sebaceous carcinoma (n=1). Metastatic tumors from distant organs (n= 18) were less common: breast (n=4), kidney (n=3), lung (n=3), thyroid (n=3), prostate (n=2), pancreas and liver (n=2), and bladder (n=1). Other tumors included small cell carcinoma (n=3), malignant peripheral nerve sheath tumor (n= 1), angiosarcoma (n= 2), rhabdomyosarcoma (n= 1), olfactory neuroblastoma (n= 1), chordoma (n=1) and unclassifiable carcinoma (n=18). Immunostaining helped to classify 36 FNA cases, including poorly differentiated SCCs and spindle cell melanomas. Surgical biopsy/resection specimens were available in 107 cases. Twenty nine (27.1%) tumors were limited to intraparotid lymph nodes (LNs). The sensitivity of SG FNA for reaching a malignant diagnosis was 86%, but decreased to 69% for a more specific classification.

Conclusions: Secondary SG malignancies are most often of cutaneous origin in older men, involving both LNs and SG parenchyma. FNA is accurate in offering diagnosis of SG malignancies. Increased awareness along with appropriate immunostaining is helpful to avoid diagnostic pitfalls.

469 FNA-Based Grading of Pancreatic Neuroendocrine Neoplasms Using Ki-67

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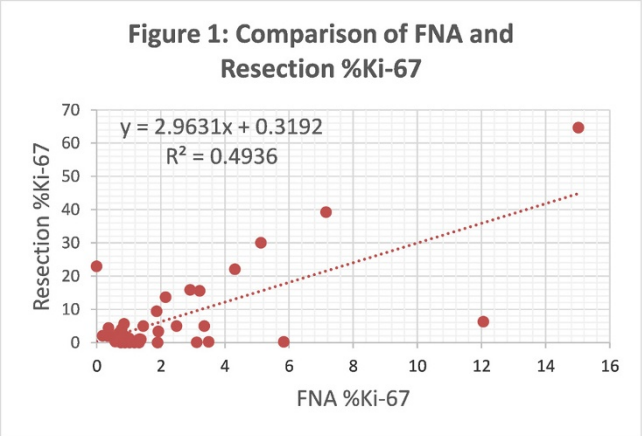
Background: Pancreatic neuroendocrine neoplasms (PanNENs) are uncommon tumors with variable clinical behavior. The most important prognostic markers are tumor grade and stage. As a result, the World Health Organization has provided grading guidelines based on mitotic count and Ki-67 proliferation index. Due to the desire to provide earlier tumor grading for clinical management decisions, some groups have proposed grading PanNENs at the time of fine needle aspiration (FNA) using Ki-67 proliferation rates. While a Ki-67 can be performed on FNA cell blocks, there are potential sampling limitations with this technique that may affect the reliability of the Ki-67 result.

Design: Thirty-eight PanNENs with FNA cell blocks and corresponding resection material were evaluated by immunohistochemistry for expression of Ki-67. Ki-67

proliferation rate was calculated based on cell counts of >500 cells in the highest staining areas. Ki-67 scores from FNA cell blocks were correlated with Ki-67 scores from resection specimens.
Results: The FNA Ki-67 proliferation rates overall were not accurate and did not correlate well with the resection specimen rates. A linear regression analysis of the correlation between FNA %Ki-67 and resection %Ki-67 showed a slope of 2.96 and an R^2=0.49 (Figure 1). The average difference in Ki-67 proliferation rate between FNA and resection was 6.2%. Thirty-nine percent (15/38 cases) of PanNENs showed discordant grading between the FNA cell block and resection specimen. All discordant cases demonstrated a lower FNA-based grade as compared to the resection grade.



Conclusions: This study provides direct evidence against using FNA cell block Ki-67 proliferation rates to determine grading. These cases showed that FNA cell block grading using Ki-67 frequently led to under-grading of the tumor. This finding is consistent with concerns that FNA may not provide accurate grading due to the limited sampling of the tumor. We recommend careful evaluation of the PanNEN resection specimen prior to providing a definitive WHO grade.

470 Metastases from Extrapulmonary Sites to Mediastinal Lymph Nodes: A 10-Year Retrospective Review of Transbronchial Needle Aspiration Cytology in a Tertiary Care Center

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Background: Transbronchial needle aspiration (TBNA) is rapidly becoming the diagnostic modality of choice for the diagnosis of lung cancers and for evaluation of the status of mediastinal lymph nodes, substantially displacing more invasive mediastinoscopy procedures. The majority of malignancies encountered in the mediastinal lymph nodes are metastatic from the lung. However, cancers from many other organs may metastasize to this region, and the incidence of mediastinal lymph node metastasis from extrapulmonary sites is largely unknown.
Design: We retrospectively reviewed all TBNAs of mediastinal lymph nodes that were diagnosed as positive for malignancy for a 10-year period at our tertiary care center. These cases were reviewed for metastasis site of origin and the lymph node station involved.
Results: 849 TBNA cases performed for lymph node evaluation were identified. Of these, 316 (37.2%) were positive for malignancy. The vast majority of cancers, 272 (86.1%), were metastatic from the lung, 31 (9.8%) were metastases from extrapulmonary sites, 10 (3.2%) were lymphomas, and 3 (0.9%) were carcinomas of uncertain origin. The extrapulmonary sites of origin included: 6 (19.4%) renal; 6 (19.4%) head and neck (squamous cell carcinomas); 5 (16.1%) esophageal; 4 (12.9%) breast; and 3 (9.7%) prostate. One case (3.2%) each of ileal, anal, undetermined upper GI/pancreaticobiliary, urothelial, melanoma, extremity sarcoma, and thyroid primaries were identified.
Of the 272 metastases of pulmonary origin, 127 (46.7%) were adenocarcinoma, 62 (22.8%) were squamous cell carcinoma, 49 (18.0%) were small cell carcinoma, 28 (10.3%) were non-small cell carcinomas not further classified, and 6 (2.2%) were other lung primaries.
Conclusions: The incidence and cytologic features of metastases to the mediastinal lymph nodes from distant (extrapulmonary) sites have not been well documented in the literature. This study demonstrated that a significant proportion of mediastinal lymph node metastases arose from a wide variety of extrapulmonary sites of origin. Awareness of the wide range of diagnostic possibilities, the individual patient's clinical history, and of the characteristic cytologic features of non-pulmonary metastases can help to prevent misdiagnosis of these tumors. Given the rate of extrapulmonary metastases to mediastinal lymph nodes identified in this study, a low threshold for immunohistochemical workup should be applied in patients with a history of prior malignancy or a non-classic presentation for lung carcinoma.

471 GATA3 Expression as a Marker for Neuroblastoma: Utility in Aspiration Cytology and Limited Tissue Samples

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Background: Neuroblastomas (NBs) are the most common solid cancer of childhood and infancy. As a "small, round, blue cell tumor," the histomorphology is non-specific and poorly differentiated cases often require immunohistochemistry (IHC) studies. Most associated with mammary and urothelial differentiation, GATA3 is implicated functionally in NB differentiation. While prior data using resections support the utility of GATA3 as part of an IHC panel for NB diagnosis, tissue samples are often limited and prioritized for evolving molecular tests. Thus, high performance diagnostic markers are necessary. Herein, the utility of GATA3 IHC using cytology aspirates and other minute biopsies is tested.
Design: We tested GATA3 IHC on eighteen consecutive cytopathology cell blocks (n=5), scant surgical biopsies and 2mm NB tissue cores (n=13) from our institution to validate the utility of this marker. IHC was performed per our standard, CLIA-validated, automated staining protocol. Samples were qualitatively graded for nuclear staining as: 0 (no staining), 1 (weak staining), 2 (moderate), 3 (strong).
Results: Of the eighteen total cases, 100% of NB showed diffuse nuclear positivity with GATA-3. Each sample revealed either strong (17 cases) or moderate (1 case) nuclear staining in histopathologically identifiable small, round NB cells, regardless of the presence or lack of stroma, necrosis, or differentiation.
Conclusions: GATA3 expression is diagnostically reliable and can be a high performance marker for NB in the setting of cytology and other minute samples, including in scant material from cases with poor differentiation. This marker is recommended to cytopathologists for diagnostic use in this often challenging setting.

472 Prior Pap Test and/or HPV Testing Results in 3342 Women with Histologically Diagnosed Cervical Intraepithelial Neoplasia 2/3: Data from China's Largest CAP Certified Clinical Laboratory

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Background: High grade squamous lesions caused by persistent hrHPV infection are regarded as precursor to cervical cancer. We examine Pap cytology and hrHPV testing results preceding histologic diagnoses of CIN2/3 in China.
Design: Cases of CIN2/3 diagnosed from 2011 to 2014 by histology were retrieved. Prior hrHPV and Pap cytology results in the year before CIN2/3 diagnoses were recorded.
Results: 3342 patients of CIN2/3 (39.0 years) had prior HC2 HPV testing and/or Pap test results including 1657 with hrHPV testing (average 1.3 months; 0.5-9) and 2369 with Pap cytology (average 1.5 months; 0.5-11). The hrHPV-negative rate was 8.8% (145 of 1657 patients) and the Pap-negative rate was 6.6% (158/2396)(p=0.01). The negative Pap rate was significantly different depending on the preparation, highest in Liqui-Prep and lowest in Lituo. Abnormal Pap test results are listed in table 1. Of 711 patients with both HPV and Pap testing results, 62 (8.7%) had negative Pap cytology and 50 (7.0%) had negative HPV testing (p= 0.23). Only 16 (2.3%) had double negative results.
Conclusions: This study demonstrates relatively high prior negative testing results (HPV and Pap test) in a population of women with CIN2/3 in China where there is no national cancer screening program. hrHPV testing was not more sensitive than Pap cytology in detection of HSIL lesion. Double negative rate is very low, supporting the value of contesting to enhance detection of cervical cancer precursors.

	ThinPrep	SurePath	Liqui-Prep	Lituo	Conventional	Total
HSIL	514 (42.1)	108 (14.6)	31 (36.5)	244 (51.7)	173 (43.7)	1070 (44.7)
LSIL	231 (18.9)	61 (27.5)	16 (18.8)	109 (23.1)	83 (21.0)	500 (20.9)
ASC-H	192 (15.7)	17 (7.7)	14 (16.5)	64 (13.6)	67 (16.9)	354 (14.8)
ASC-US	176 (14.4)	20 (9.0)	11 (12.9)	42 (8.9)	54 (13.6)	303 (12.6)
AGC	8 (0.7)	1 (0.5)	0	1 (0.2)	1 (0.3)	11 (0.5)
Negative	100 (8.2)	15 (6.8)	13 (15.3)	12 (2.5)	18 (4.5)	158 (6.6)
Total	1221	222	85	472	396	2396

Category	HPV Positive		HPV Negative		Total	
	N	%	N	%	N	%
HSIL	272	41.1	9	18	281	39.5
LSIL	146	22.1	6	12	152	21.4
ASC-H	84	12.7	6	12	90	12.7
ASC-US	110	16.6	13	26	123	17.3
AGC	3	0.5	0	0	3	0.4
Negative	46	7.0	16	32	62	8.7
Total	661	100	50	100	711	100

473 Refine Risk of Malignancy by Subclassifying Atypia of Undetermined Significance (AUS) in Thyroid Cytopathology

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Background: The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is to standardize the terminology and to improve implication of risk of malignancy (RM) of thyroid nodules. AUS of TBSRTC is a heterogeneous group with implied RM 5-15%. Subclassification of AUS cytology is likely to improve implication of RM. This study is to investigate the implication of RM of thyroid nodules with AUS cytology.
Design: A retrospective search PowerPath database was conducted from 07/2013 to 07/2015. The thyroid AUS cases were reviewed and sub-classified into five categories

by adapting Mathur's criteria (SURGERY 156:1471, 2014): focal nuclear atypia (AUS-N), focal microfollicular proliferation (AUS-F), focal Hürthle cell proliferation (AUS-H), focal nuclear atypia mixed with focal microfollicles and/or focal Hürthle cell proliferation (AUS-M), and others (AUS-O). The pathology diagnosis of resected thyroid nodules with prior AUS was reviewed and the RM was assessed for following malinancy: papillary thyroid carcinoma (PTC), follicular carcinoma (FC), follicular variant papillary carcinoma (FVPTC), and others.

Results: Total 36 out of 79 AUS cytology cases have surgical resection of thyroid nodules. The overall RM is 36.1% (13/36). The individual RM rate of resected thyroid nodules with AUS cytology of AUS-N, -F, -HC, -M and -O is 43.5%, 100%, 25%, 20%, 0%, respectivel. AUS-N and -F has much higher implied RM than those of AUS-HC, -M and -O, which have RM near the upper limit range defined by TBSRTC.

TBSRTC cytology	**Benign**		**Malignant**	
(No.=36)	**No. of specimens**	**Percentage (%)**	**No. of specimens**	**Percentage (%)**
AUS-N (No.=23)	13	13/23=56.5%	10 (5 PTC, 5 FVPTC)	10/23=43.5%
AUS-F (No.=1)	0	0/1=0%	1 (1 FVPTC)	1/1=100%
AUS-HC (No.=4)	3	3/4=75%	1 (1 FVPTC)	1/4=25%
AUS-M (No.=5)	4	4/5=80%	1 (1 FC)	1/5=20%
AUS-O (No.=3)	3	3/3=100%	0	0/3=0%

Conclusions: Thyroid cytology of AUS-N and -F carries higher RM rate than those of AUS-HC, -M and -O. Our data suggest that, based on implied RM rates, the AUS of TBSRTC can be further classified into high-risk group (AUS-N and -F) and low-risk groups (AUS-HC, -M and -O). The subclassification will improve stratification of RM of thyroid nodules with AUS cytology. Further large-scale studies are warranted to consolidate our conclusion.

474 Cytology and High-Risk HPV DNA Co-Testing in Cervical Cancer Screening Program: Outcome of 3-Year Follow-Up in an Academic Institution

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Background: Cytology and high-risk HPV DNA (hrHPV) testing, Co-testing, has been increasingly used in cervical cancer screening. The present study summarized the outcome of Co-testing by reviewing up to 3 years of clinical and pathological follow-up information.

Design: Patients with Co-testing during 2012 to 2013 were retrospectively identified and their electronic medical records were reviewed. Follow-up information, including subsequent histology, cytology, and clinical information were collected. In cases with multiple histology and cytology results during the study period, the worst result was recorded. In cases without histology or cytology follow-up information, patients were marked as No Screening or No Record if they continued under a physician's care or were lost to follow-up, respectively. All histologically proven HSIL cases that were interpreted as NILM by cytology were reviewed.

Results: Results of 2555 patients with Co-testing were obtained, including 1575 patients with NILM)/HPV -, 141 with NILM/hrHPV+, 502 with ASCUS/hrHPV-, 274 ASCUS/hrHPV+, and 66 with cytology LSIL or worse. The follow-up information is shown in the Table. There were 6 histologically proven HSIL cases in which cytology interpreted as NILM and hrHPV was positive. In reviewing these cases, 2 remained as NILM, and the other 4 were reclassified as HSIL, ASC-H, and 2 ASCUS, respectively. There was 1 case in which hrHPV was negative and both cytology and biopsy showed HSIL. A total of 22 histologically proven LSIL was negative for hrHPV.

Group	Histological Follow-up			Cytologic Follow-up				Other	
	Benign	LSIL	HSIL	NILM	ASCUS	LSIL	HSIL	No record	No Screening
NILM/ hrHPV+ (n=141)	7	15	6	43	8	3	0	39	20
ASCUS/ hrHPV- (n=502)	39	16	0	151	21	8	0	106	161
ASCUS/ hrHPV+ (n=274)	32	86	27	36	18	16	1	38	20
LSIL/ hrHPV- (n=17)	1	6	0	5	2	0	0	3	0
LSIL/ hrHPV+ (n=29)	4	9	4	5	1	1	0	2	3
ASC-H/ hrHPV- (n=3)	2	0	0	0	0	0	0	0	1
ASC-H/ hrHPV+ (n=11)	1	0	6	0	0	0	0	2	2
HSIL/ hrHPV- (n=1)	0	0	1	0	0	0	0	0	0

Conclusions: hrHPV DNA testing rarely misses HSIL, although a significant number of LSIL were hrHPV negative. Co-testing is an optimal method to identifying patients with a higher risk for developing histologically proven cervical abnormalities.

475 Refined Diagnostic Criteria for the Cytopathologic Diagnosis of Mucinous (Colloid) Adenocarcinoma of the Pancreas

Xiu Yang, Houda Alatassi, Mostafa M Fraig. University of Louisville, Louisville, KY.

Background: Diagnosis of colloid carcinoma of the pancreas can be difficult in view of the usual low cellularity and bland cytological features. Frequently, these cases are underdiagnosed. We analyzed a cohort of cases with the proven diagnosis of colloid denocarcinoma

for the most prevalent diagnostic criteria in comparison to well differentiated ductal adenocarcinoma, which is another difficult diagnosis.

Design: A computer-based search of our files spanning the period from January, 2010 to September, 2015 identified 16 cases with a diagnosis of mucinous adenocarcinoma. Three cytopathologists evaluated these cases based on 10 criteria and scored them for their presence or absence. These criteria included: 1- Thick cellular mucin, 2- Intracytoplasmic mucin indenting the nucleus, 3- Degenerative changes and necrosis, 4- Small clusters with community borders, 5- Sheets with honeycomb pattern, 6-Three-dimensional fronds, 7- Nuclear enlargement, 8- Nuclear membrane irregularity, 9- Confluence and gaps among nuclei, 10- Nuclear pleomorphism.

Results: The most prevalent features in our cohort were the first 6 criteria with thick mucin present in all cases (16/16), intracytoplasmic mucin in 13/16, and the other three present in 11/16. Sheets with honeycomb pattern are present in 13/16 cases. Other common criteria in well differentiated adenocarcinoma; nuclear membrane irregularity was present in 8/16, pleomorphism in 5/16, confluence and gaps of nuclei in 4/16, nuclear enlargement in 3/16.

Conclusions: Additional criteria could help reach a definitive in otherwise hypo-cellular specimens and bland appearing cells from cases of colloid carcinoma of the pancreas. The histopathologic diagnosis is usually aided by the presence of dissecting mucin and floating cellular clusters. In cytologic preparation, the presence of thick mucin admixed with macrophages and degenerated cells, small clusters with smooth community borders and intracellular mucin indenting the nucleus are additional criteria to add to criteria used in the diagnosis of well differentiated ductal adenocarcinoma of the pancreas. The presence of the three dimensional papillary fronds could point to a precursor intraductal papillary mucinous neoplasm (IPMN). Evaluating larger cohorts for these criteria may be helpful in further validating them.

476 MicroRNA Profiling of Malignant Mesothelioma: Diagnostic Significance in Cytology Specimens

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Background: Malignant mesothelioma (MM) is a rare but aggressive cancer. One contributing factor to poor prognosis is the difficulty to diagnose this entity at an early stage due to the challenge of differentiating MM from reactive mesothelial cells and of demonstrating the invasion of MM on cytology specimens. Currently there are no generally accepted diagnostic biomarkers for MM. MicroRNAs (miRNAs) regulate the expression of target mRNAs at transcriptional and translational levels and are differentially expressed in neoplastic compared to normal tissues. The ability to detect miRNA expressions in body fluids has generated interest in their possible role as tumor biomarkers on cytology specimens. Therefore, it is essential to develop a more sensitive method to detect MM cells. The purpose of this study is to identify miRNA profiling in MM and its diagnostic significance in cytology specimens.

Design: 1) Detect the miRNA expression profiles of MM and normal mesothelial cell lines using Human Cancer PathwayFinder miRNA PCR Array.

2) Select miRNAs upregulated in MM as target miRNAs for this study after comparing miRNA expression profiles of MM and normal mesothelial cells.

3) Confirm the differential expressions of target miRNA in MM and reactive mesothelial cells on surgical specimens (6 MM, 7 reactive) by in-situ hybridization (ISH).

4) Test the expressions of miRNAs in cytology specimens (4 MM)by ISH.

Results: The miRNA PCR Array reveals different expressions of 84 miRNAs in MM and normal mesothelial cell lines. Five miRNAs (miR-32, miR-96, miR-135b, miR-148a, and miR-183) are found to be significantly upregulated in MM cell lines. Among the five miRNAs, miR-135b, miR-148a, and miR-183 are expressed on selected MM surgical specimens by ISH, with miR-183 expression the strongest. Reactive mesothelial cells in selected surgical cases do not express miR-135b or miR-148a and weakly express miR-183. miR-183 is strongly expressed in all 4 MM cytology specimens. miR-135b and miR-148a are expressed in 2 MM cytology specimens.

Conclusions: This study shows that miR-183 has strongest expression in all surgical and cytology MM cases. miR-135b and miR-148a are expressed in all surgical MM cases and some of the cytology specimens. In comparison, benign mesothelial cells do not express miR-135b or miR-148a and only weakly express miR-183 on the surgical specimens. The above findings suggest that these miRNAs, especially miR-183, could be a potential biomarker for diagnosing MM on cytology specimens.

477 Triaging Cases with Clinically Suspicious Nodules and Negative Findings by Electromagnetic Navigational Biopsy: Experience from One Tertiary Care Center

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Background: Peripheral pulmonary nodules (PPN) are common radiological findings which can be challenging to diagnose by the conventional image-guided transthoracic or bronchoscopic biopsy. The Electromagnetic Navigation Bronchoscopy (ENB) is a

relatively new technique for peripheral lung lesions sampling with superior safety profile and cost effective approach. We hereby present our experience in triaging clinically suspicious cases with negative histopathological findings post ENB biopsy.
Design: A computer based search of our institution's database revealed 62 cases who underwent ENB biopsy. The search covered the period from March 2012 To March 2015. The patients' medical records were reviewed as well as the cytopathologic and histopathologic findings whenever available. Six cases did not have sufficient follow-up at time of the study.
Results: Mean age of patients is 61.4 years, male: female ratio is 1:1 in general and 3:1 among patients with malignant diagnoses. On the initial ENB biopsy, thirteen cases were diagnosed as positive for malignancy and 43 cases had negative diagnoses. Among the latter group and after 3-36 months follow up, 7 cases converted to positive for malignancy. Of those, 5 cases have pathologic confirmation. Two other cases developed metastatic cancer shortly after ENB. Four initially negative cases underwent repeat biopsy and remained negative for malignancy. Thirty two cases were followed up radiologically with no evidence of progression during their follow up period. Of the total 20 cases positive for malignancy, 15 cases have primary lung carcinomas (6 cases with adenocarcinoma, 5 cases with squamous cell carcinoma, and 4 cases with non-small cell carcinoma) while 5 cases have metastatic tumors to the lung.
Conclusions: Based on the literature and on our study, ENB biopsy has proven its utility in providing accurate and cost effective diagnosis in evaluating PPN. As with any diagnostic technique, the negative results can be a mixture of possibilities. Our data supports the use of different approaches in triaging these cases. This ranges from radiological and clinical follow up to repeat attempts of biopsy. Only a minority of cases (9/62) benefitted from repeat studies, especially when the radiological and clinical index of suspicion is high. A larger study to confirm these findings would be very helpful.

478 Comparative Evaluation of the Paris Reporting System for Urine Cytology with Our Original Urine Cytology Reporting System

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Background: Urinary Tract Cytology (UTC) is currently the most commonly used test for screening and monitoring of urothelial carcinomas. The proposed Paris System for Reporting Urine Cytology is being designed to standardize the criteria and terminology used in UTC reporting. The aim of this study is to compare and correlate the Paris Reporting System (PRS) to our institution's Original Reporting System (ORS) with follow up biopsy results.
Design: Two hundred UTC cases with follow-up biopsies from 2010 and 2011 were selected for evaluation. The biopsy diagnoses were classified based on the 2004 WHO / ISUP classes, with 110 cases being neoplasms (52 Low Grade Urothelial Neoplasms or LGUN, 51 High Grade Urothelial Carcinomas or HGUC, and 7 other malignancies) and 90 being negative for malignancy.
The corresponding 200 UTC cases initially diagnosed by ORS, were re-classified independently and blindly by two senior cytopathologists according to the proposed PRS. By our ORS criteria the UTC cases were reported as: Negative (n=116), Urothelial Atypia (UA) (n=45), LGUN (n=4), suspicious for HGUC (n=4), HGUC (n=28), and atypia-other malignancies (n=3). By proposed PRS categories, cases were classified as: 1=Unsatisfactory (n=5), 2=Negative for HGUC (n=124), 3=Atypical urothelial cells of uncertain significance (n=24), 4=Atypical urothelial cells suspicious for HGUC (n=9), 5=HGUC (n=32), 6=LGUN (n=3), and 7=other malignancies (n=3). Statistical analysis was performed using SPSS.
Results: Comparing UTC to biopsy, the sensitivity of detecting urothelial neoplasms was higher in PRS (42.7%, 47/110) than ORS (32.2%, 36/110). Atypia was diagnosed more frequently in ORS (n=48) than PRS (n= 24). Out of 51 HGUC cases, PRS detected 33 (64.7%) while ORS detected 27 (52.9%). Out of 52 LGUN, ORS detected 4 and PRS detected 3 cases. Of 7 other malignancies, both ORS and PRS classified 6 as abnormal UTC (either
LGUN, suspicious for HGUC, HGUC or others). Both UTC reporting systems showed overall good correlation and concordance with biopsy diagnosis ($p<0.001$), with PRS demonstrating slightly better results ($R=0.604$, $\kappa=0.341$) than that of ORS ($R=0.561$, $\kappa=0.251$).
Conclusions: Use of PRS reduced the rate of atypia diagnosis by 50%.
PRS showed improvement in detecting urothelial neoplasms, especially HGUC.
Both ORS and PRS detected only few cases of LGUN.
PRS was the same as ORS in detecting other non-urothelial malignancies
Overall, PRS showed improved correlation and concordance with histopathologic results comparing to ORS.

479 Bethesda System Reporting of Benign-Appearing Endometrial Cells in Women 40 and Older: Analysis of Predictive Value from a Large Academic Women's Hospital Database

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Background: The Bethesda System (TBS) 2001 introduced Pap test reporting of benign-appearing endometrial cells (nEMC) in women 40 and older and replaced TBS 1991 reporting of nEMC only in postmenopausal women. This change was made because menopausal status was often unclear, inaccurate, or unknown to the laboratory. TBS 2015 further slightly modified reporting of nEMC to women 45 and older "to improve the predictive value of exfoliated benign-appearing EMC." Many unnecessary endometrial procedures have resulted, despite continued absence of published evidence of improved clinical outcomes.
Design: A 10 year database containing over 1,000,000 Pap test reports and over 100,000 surgical pathology reports identified 3361 cases with Pap reports of nEMC in women 40 and older and follow-up histopathologic endometrial reports within 6 months. Atypical endometrial hyperplasia (AEH) and carcinoma (EmCa) were chosen as clinically relevant abnormal endpoints. Patients were stratified into 5-year age groups from 40 to ≥ 60. Negative predictive value (NPV) and number of cases with abnormal histopathologic endpoint findings were analyzed.
Results: AEH and EmCa were identified in < 1% of patients ages 40-49. There was no statistically significant difference in NPV between these two groups. A significant decrease in NPV was detected among patients 50 and older. No statistically significant difference was noted between subgroups of women 50- ≥ 60. Among 1869 women ages 40-49, only 2 asymptomatic patients (0.1%) had follow-up diagnoses of either AEH or superficial low grade EmCa.

Benign-Appearing Endometrial Cells with Surgical Follow-Up within 6 Months			
Age	Number of Cases	AEH or EmCa, n (%)	NPV
40-44	886	5 (0.56)	99.44%
45-49	983	8 (0.81)	99.19%
50-54	658	19 (2.89%)	97.11%
55-59	391	17	95.65%
≥60	443	25	94.36%

p-value for comparison of NPV among age groups				
	40-44	45-49	50-54	55-59
45-49	0.5149			
50-54	0.0003*	0.0012*		
55-59	<.00001*	<.00001*	0.2093	
≥60	<.00001*	<.00001*	0.0223	0.3952
* statistically significant difference (two-sided z-test)				

Conclusions: TBS reporting of nEMC in women 40 and older has often been misinterpreted as an abnormal cytologic finding with significant positive predictive value, a misunderstanding that has led to many potentially harmful endometrial procedures, despite an absence of proven clinical benefit.

480 High Diagnostic Accuracy of Concordant Ucyte and Urovysion Tests

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Background: Ucyte and Urovysion are two technologies to detect presence of urothelial cancer. The sensitivity and specificity are both affected by stage and grade of the tumor. The aim of this study is to compare the performance of the two tests in the same patient and evaluate whether the concordant results have higher diagnostic accuracy.
Design: Fourty-four patients with concurrent Ucyte and Urovysion tests were retrospectively selected. The test results were compared to histology diagnosis, or consistent cytology and cystoscopy findings.
Results: The sensitivity, specificity, positive predictive value, negative predictive value for Ucyte and Urovysion are 0.75/0.53/0.38/0.85 and 0.67/0.62/0.4/0.83 respectively ($p>0.05$). The diagnostic accuracy is 59% for Ucyte and 64% for Urovysion. The concordance rate for Ucyte and Urovysion is 73% (32/44). For the concordant Ucyte and Urovysion results, the diagnostic accuracy is 100%.
Conclusions: Our result suggests comparable performance between Ucyte and Urovysion; and that when the results of the two tests are concordant, the diagnostic value is high.

481 Cytomorphologic Features and EGFR Mutational Status of Combined Small Cell Lung Carcinomas

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Background: Combined small cell lung carcinomas (CSCLC) are rare and defined by mixed components of small cell carcinoma and non-small cell carcinoma. The non-small cell carcinoma components may include squamous cell carcinoma, adenocarcinoma, large cell neuroendocrine carcinoma, and/or less commonly sarcomatoid carcinoma. These mixed histologic patterns may present a diagnostic challenge in cytology specimens. The aim of study is to investigate the cytomorphologic features of CSCLC and their *EGFR* mutational status.
Design: A database search was conducted for surgical biopsy and resection specimens from January 2009 to August 2015 for the keywords "combined small cell carcinoma". For the cases with combined small cell carcinoma, a search for concurrent cytology sample was performed. The clinical presentation, the pathologic reports, and *EGFR* molecular analysis were reviewed.
Results: Twenty-five (25) lung specimens (9 biopsies, 16 resections) with combined small cell lung carcinoma were reviewed. The non-small cell carcinoma components in those 25 cases were large cell neuroendocrine carcinoma (16), adenocarcinoma (4), adenosquamous carcinoma (1), squamous cell carcinoma (2), carcinosarcoma (1), and non-small cell carcinoma, unclassifiable (NSCLC, NOS) (1). Of those, 18 patients also had a cytology specimen from the same tumor. Cytology interpretations of those 18 cases were combined small cell carcinoma (6), small cell carcinoma (7), large cell neuroendocrine carcinoma (2), adenocarcinoma (1), and NSCLC- NOS (2). *EGFR* mutation analysis was performed on 8 of those cases with two positive results (25%): *EGFR* exon 19 in-frame deletion (1) and G719X (1). These two patients had a prior diagnosis of adenocarcinoma with positive *EGFR* mutation and were treated with EGFR inhibitors.
Conclusions: Diagnosing combined small cell carcinoma from cytology sample could be challenging due to the sampling issue. Combined small cell carcinoma can be

associated with *EGFR* mutation as a result of histologic transformation from non-small cell carcinoma to small cell carcinoma. Our findings suggest that *EGFR* mutations should be tested in combined small cell carcinoma.

482 PCR Human Papillomavirus Detection in Invasive Cervical Cancers with Prior Negative HC2 Test Results

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Background: Though HPV infection is accepted as a necessary cause of invasive cervical cancer, accumulating data indicate that a portion of invasive cervical cancers have negative HPV test results in preceding cytology. In two recent large studies using data from China, 15.5% and 7.5% cervical cancer patients had recent prior HPV testing. The current study investigates whether hrHPV DNA is detectable in the resected cervical cancer tissue from those cases with prior negative HC2 results.

Design: 61 cervical cancer patients (50 squamous cell carcinomas, 8 adenocarcinomas, and 3 adenosquamous carcinomas) with prior negative HC2 results from the prior two studies (most in the 3 months) were included. Paraffin blocks were processed under strict conditions to avoid contamination. DNA was extracted from 10 paraffin sections from each block. In order to increase the sensitivity, PCR with three consensus primer sets (GP5+/6+, MY09/11, SPF1/2) for different L1 regions of viral genome was performed for HPV detection.

Results: The average age of the patients was 47 years (range 27-78). 13 cases were positive by PCRs with all three primer sets and one case was positive by GP5/6 and SPF1/2. All other 47 cases were negative by PCR with all three primer sets (77%). The overall HPV detection rate in these tumors was 23% (14/61). Prior Pap cytology results associated with positive PCR HPV testing in tissue samples are shown in table 1. HPV DNA was not detected in all eight cases of adenocarcinomas including 2 minimal deviation carcinoma and 1 clear cell carcinomas.

Conclusions: This is the first study to exam the ability to detect HPV DNA by PCR in cervical cancer tissue from patients with prior negative HC2 testing. HPV was detectable in only 23% of these resection specimens. These findings suggest that cervical carcinomas with undetectable HPV DNA are the primary reason for so called "false negative" screening HPV test results and that this is an important limitation in the potential use of primary HPV screening.

Prior Pap	PCR Tested	PCR Positive	Percentage
SCC	8	3	37.5
HSIL	17	4	23.5
ASC-H	5	2	40
AGC	4	0	0
ASCUS	3	1	33.3
NILM	7	2	28.6
N/A	17	2	11.8
Total	61	14	23.0

483 Comparing the Histological Follow-Up Results between Positive HC2 HR HPV Test and Cervista HPV HR Assay for Women with ASC-US Cytology in a large Academic Women's Hospital Practice

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Background: Data remain very limited comparing histological follow-up results for women with ASC-US when HPV testing is performed using different FDA-approved methods.

Design: A computer-based search was carried out to retrieve the cases with ASC-US Pap tests and positive HC2 HPV result from 6/2012 to 5/2013 and the cases with ASC-US Pap tests and positive Cervista HPV HR result from 6/2013 to 5/2014. Histological follow-up results were analyzed.

Results: 3317 cases with positive HC2 HPV/ASC-US Pap tests and 4297 cases with positive Cervista HPV HR/ASC-US Pap tests were found. The first continuous 1500 cases in each group were checked for the histological follow-up results. The average age was 33.7 years in HC2 HPV group (50% ≥30 years) and 36.9 years in Cervista HPV HR group (60.8% ≥30 years). Total 1700 cases had histological follow-up within one year peroid. The average initial follow-up period was 2.1 months (0.1-12 months). Overall CIN2/3 was detected in 6.4% cases and CIN1+ lesions were detected in 45.7% cases. CIN2/3 and CIN1 detection rates in HC2 HPV group were significantly higher than that in Cervista HPV HR group ($p<0.01$).

Conclusions: This is one of the largest study to compare HC2 HPV testing and Cervista HPV HR testing results for women with ASC-US Pap cytology. The results demonstrate that detection rates of both high and low grade cervical squamous lesions are significantly higher in ASC-US/positive HC2 HPV group compared with ASC-US /positive Cervista HPV HR group. The fact that more percentage of women <30 years in HC2 group than in Cervista HPV HR group may partially explains the difference. Additional studies on positive and negative predictive values, sensitivity, and specificity are needed to fully assess differences in HPV test performance.

HR HPV test	Age	Total case#	F-u#	CIN2/3 (%)	CIN1 (%)	Negative (%)
HC2	<30	750	401	42 (10.5)	198 (49.4)	161 (40.1)
	≥30	750	476	32 (6.7)	173 (36.3)	271 (56.9)
	sub-total	1500	877	74 (8.4)	371 (42.3)	432 (49.3)
Cervista	<30	588	204	16 (7.8)	91 (44.6)	97 (47.6)
	≥30	912	619	18 (2.9)	207 (33.4)	394 (63.7)
	sub-total	1500	823	34 (4.1)	298 (36.2)	491 (59.7)
Summary	<30	1338	605	58 (9.6)	289 (47.8)	258 (42.6)
	≥30	1662	1095	50 (4.6)	380 (34.7)	665 (60.7)
	Total	3000	1700	108 (6.4)	669 (39.4)	923 (54.3)

484 High Risk HPV Testing and Report Rate: Result from the Largest CAP Certified Independent Laboratory in China

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Background: Reports of hrHPV testing patterns and positive rates in different cytological categories from China are rare. We evaluated testing patterns and positive rates in different cytological categories in China's largest CAP-accredited laboratory.

Design: Results from 128,195 Pap tests with HC2 HPV testing result, rendered between 2011 and 2014 in the lab were analyzed. The samples for Pap test and HPV test were saved in two different vials.

Results: The hrHPV-positive rate was 35% in patients for ASC-US, with 40% in patients <30 years and 34.1% in patients >=30 years. The hrHPV-positive rate was 12.1% for NILM, with 14.6% in patients <30 years and 11.5% in patients >=30 years. The overall hrHPV-positive rates were 77.7% in LSIL, 90.5% in HSIL and 80.8% in ASC-H and 47% in AGC. The hrHPV-positive rate was similar in various liquid-based cytology methods including ThinPrep, SurePath, LITOU liquid-based preparation, but higher in conventional and LIPU preparations.

Conclusions: This is the first routine clinical practice report of hrHPV positive rates in variable Pap cytology categories. The hrHPV-positive rate reported from China's largest CAP-accredited laboratory was comparable to that reported among US laboratories (CAP 2012 National Survey Arch Pathol Lab Med 2015;139:757–761). Therefore, participation in the international CAP Laboratory Accreditation Program provides laboratory quality standards not otherwise available in many international settings. HPV positive rate is 12% for NILM, much higher than that in most reports in the Western countries, indicating higher prevalence of hrHPV infection in Guangdong, China.

Categories	<30 years		>=30 years		Total		
	Case#	Positive (%)	Case#	Positive (%)	Case#	Positive (%)	Ages
ASC-US	2425	970 (40.0)	11239	3827 (34.1)	13664	4797 (35.1)	37.8 (16-80)
LSIL	1325	1032 (77.9)	4339	3367 (77.6)	5664	4399 (77.7)	35.8 (15-93)
ASC-H	63	43 (69.3)	671	550 (82.0)	734	593 (80.8)	43.7 (23-80)
HSIL	78	64 (82.1)	1157	1054 (91.1)	1235	1118 (90.5)	42.5 (16-80)
AGC	12	6 (50.0)	122	57 (46.7)	134	63 (47.0)	41.5 (28-64)
NILM	19237	2807 (14.6)	87527	10065 (11.5)	106764	12872 (12.1)	xx
Total	23140	4922 (21.3)	105055	18920 (19.0)	128195	23842 (18.6)	xx

485 Challenge of Fine Needle Aspiration Diagnosis of Angiomyolipoma: A Study of 33 Cases

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Background: Angiomyolipoma (AML), typically composed of smooth muscle, vessel and fat, is generally a benign tumor in the kidney. It can occasionally occur in extra-renal sites and behave like a malignant tumor. AML is uncommonly encountered in cytology practice and can cause diagnostic difficulty.

Design: We searched our pathology database between 2003 and 2015 for fine-needle aspiration (FNA) cases that were histologically confirmed to be AML, and retrospectively reviewed diagnostic accuracy and cytology features.

Results: Thirty-three FNA cases from 31 patients (9 male and 22 female) were identified; one patient had three FNA cases. Age at the time of cytology diagnosis ranged from 31 to 83 years old (mean, 58 years old). The aspiration sites included kidney (28),

liver (3), abdominal wall (1) and lung (1). Of the 33 FNA cases, 30 were primary AML (28 in kidney and 2 in liver) and 3 were metastasis (in liver, lung and abdominal wall, respectively). FNA diagnoses included consistent with or favor AML (16, 49%), descriptive (13), non-diagnostic (1) and erroneous diagnosis (3). Of the latter 3 cases (all in the kidney), two were called "clear cell renal cell carcinoma" due to predominant epithelioid component and one was called "pleomorphic malignancy". Two renal AMLs had co-existing metastatic carcinoma (neuroendocrine carcinoma from pancreas and metastatic lung adenocarcinoma, respectively).
Upon review, smooth muscle component was most commonly seen (19), followed by vascular component (17) and adipose tissue (6). Only 4 cases showed all the three components; 13 cases had 2 components and 7 cases had smooth muscle component only. Fifteen cases showed epithelioid smooth muscle component, 7 of which were predominantly epithelioid including all extra-renal cases (4) and erroneous cases (3). Eight of the 13 cases with descriptive diagnosis have epithelioid component. Of the 33 FNA cases, 19 had contributory cell block and 9 had smear only. Immunostains were performed in 11 cases (10 on cell block and one on smear) and led to AML diagnosis in 9 cases. Positive expression of HMB45 was found in 8 of 10, Melan-A in 4 of 4 and SMA in 4 of 4 cases. No immunostaining was performed on the 3 erroneous cases.
Conclusions: FNA diagnosis of AML may be challenging, especially when it has extra-renal location (as primary or metastatic) and/or shows epithelioid component. Immunostaining is important to improve diagnostic accuracy of this rare entity.

486 Next Generation Sequencing of NSCLC in EBUS-FNA vs. Corresponding FFPE Samples

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Background: Next Generation Sequencing (NGS) on endoscopic ultrasound-guided fine needle aspiration (EBUS-FNA) samples is useful for facilitating treatment decisions of non-small cell lung cancer (NSCLC) patients. We assessed the effectiveness of detecting driver mutations by NGS in surgical and biopsy tissue versus liquid based cytology (LBC) specimens.
Design: Archived extracted DNA from residual cell pellets of LBC specimens (method previously described) with known *EGFR* and *KRAS* mutation status were retrieved. For each case, DNA from corresponding formalin-fixed paraffin-embedded (FFPE) tissue was also extracted. Mutation hotspot NGS libraries for *BRAF, EGFR, ERBB2, FGFR1, KRAS, MET*, and *PIK3C* were prepared on all DNA samples, sequenced by Illumina MiSeq and analyzed using NextGENe software with reference genome hg19. Each patient's NGS variant calls were compared between FFPE and LBC results. *EGFR* and *KRAS* results were also compared against prior real-time PCR results (Qiagen).
Results: Ten patients from June to August 2014 had adequate DNA in both LBC and FFPE specimens as measured by Qubit™ fluorometric quantitation. LBC samples were obtained from EBUS-FNA (9) or bronchial washing (1). Ten corresponding FFPE tissue specimens (6 transbronchial biopsies, 2 lobectomies, 1 each of brain and bone metastases) were selected for sequencing.

Pathologic Diagnosis	Mutation Status	Cytology Specimen	FFPE Tissue Specimen
Poorly differentiated adenocarcinoma	*KRAS*-	EBUS-FNA	Transbronchial Biopsy
Adenocarcinoma with solid (70%) and acinar (30%) patterns	c.34G>T p.Gly12Cys	EBUS-FNA	Transbronchial Biopsy
Adenocarcinoma, solid predominant	*KRAS*-	EBUS-FNA	Lobectomy
Adenocarcinoma	*KRAS*-	EBUS-FNA	Transbronchial Biopsy
Adenocarcinoma	c.35G>A, p.Gly12Asp	Bronchial Washing	Transbronchial Biopsy
Adenocarcinoma	c.34G>A, p.Gly12Ser	EBUS-FNA	Transbronchial Biopsy
Poorly differentiated NSCLC favor adenocarcinoma	*KRAS* - *ALK* t(2p23) by FISH	EBUS-FNA	Transbronchial Biopsy
Poorly differentiated w/ squamous differentiation	*KRAS*-	EBUS-FNA	Tibial Reamings
Adenocarcinoma, solid predominant	c.34G>T, p.Gly12Cys	EBUS-FNA	Lobectomy
Poorly differentiated adenocarcinoma	*KRAS*-	EBUS-FNA	Brain Excision

The average DNA concentration for LBC samples was 43.2 ng/µl and FFPE was 12.3 ng/µl. Mean average read depths were 10,899 and 7,980 for LBC and FFPE, respectively. Five FFPE and 1 LBC specimens had minimum read depths below quality control standards (<100) involving primarily *PIK3CA* (6) and *BRAF* (3).
Targeted genes were concordant across specimen types. For both FFPE and LBC, NGS detected *KRAS* mutations previously identified by PCR. In addition, all 20 specimens showed a common *EGFR* polymorphism c.2361G>A.
Conclusions: NGS results of FFPE and LBC (DNA from cell pellets) were consistent, but the superior coverage depth of LBC specimens supports its clinical use. Our initial experience using NGS on 128 clinical lung cancer FNAs detected *KRAS* mutations in 30 patients and *EGFR* in 16; qualifying 8 for tyrosine kinase inhibitor therapy. Thus, the clinical utility of NGS on EBUS-FNA derived LBC samples appears promising.

Dermatopathology

487 Validation of a New SNP-Array Platform as an Ancillary Tool for the Diagnosis of Difficult Melanocytic Lesions

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Background: Atypical melanocytic tumors that defy classification as benign or malignant using pathological criteria alone are not uncommon. Additional ancillary tests are often needed to enhance diagnostic accuracy. Prior studies have shown that evaluation of copy number variations (CNV) by array comparative genomic hybridization can be used to assist in differentiating nevi from melanomas. The performance of this technique in ambiguous melanocytic lesions and its correlation with clinical outcomes are yet to be fully characterized. More recently, platforms based on single nucleotide polymorphism arrays (SNP) arrays have become available; however, experience with their use in melanocytic lesions is limited. Herein we aim to evaluate the performance of a SNP-array on a cohort of benign, malignant and ambiguous melanocytic tumors with clinical follow-up.
Design: Eighty five melanocytic lesions were included. Using histopathological examination and immunohistochemistry, the cases were classified into four categories; benign nevi, atypical nevi, ambiguous lesions and melanomas. Follow-up data was gathered from our institutional electronic health record system and adverse events including sentinel lymph node metastasis, local recurrence, distant metastasis or death were recorded. Tissue from unstained slides or tissue rolls was processed and analyzed for CNV and loss of heterozygosity using the OncoScan V3 SNP-array platform (Affymetrix).
Results: Follow-up data was available in 57 patients (mean 5.9 months). 6/6 benign nevi (100%) and 12/15 (80%) atypical nevi showed no significant CNVs. None of these patients with follow up data had adverse events. 14/24 (58%) ambiguous lesions and 36/40 (90%) melanomas showed at least one significant alteration. In the ambiguous group, lymph node metastases were detected in 2/3 (66%) of patients with CNVs and 0/2 (0%) of patients without CNVs. The average number of CNVs in melanoma patients with or without adverse outcome was 23.4 versus 12.7, respectively. The average number of CNVs in primary versus metastatic lesions was 17.7 versus 24.5 respectively.
Conclusions: SNP-arrays may improve our diagnostic accuracy in the study of difficult melanocytic lesions. On a limited sample, it may show negative predictive value for lymph node metastasis in ambiguous lesions. In addition, in definitive melanomas, the total number of alterations correlates with adverse outcome.

488 Intratumoral Lymphovascular Invasion Detected by D2-40 Correlates with Metastasis in Primary Cutaneous Merkel Cell Carcinoma

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Background: Primary Cutaneous Merkel cell carcinoma (MCC) is an aggressive neuroendocrine cancer with a high frequency of metastasis and death. Lymphovascular invasion (LVI) has been shown to correlate with more aggressive phenotype in many tumor types. Here, we examined the significance of LVI detected by D2-40 immunohistochemical (IHC) stain in the prognostic assessment of PCMCC.
Design: We performed a retrospective analysis of 58 patients with PCMCC (1/09-7/14). LVI was determined by H&E and D2-40 IHC. When present, the location (peritumoral or intratumoral) and the size of the vessel were scored. These were compared to LVI by H&E and association with demographic, histologic, Merkel cell polyomavirus status (MCPv) and clinical outcome parameters.
Results: Fifty-eight PCMCC were assessed for LVI using H&E and D2-40. D2-40 increased the detection rate of LVI compared to H&E alone: 44 detected by D2-40 IHC vs. 30 by H&E. D2-40 IHC detected 14 cases missed by H&E, and identified 7 false positive cases by H&E. Histologically the infiltrative growth pattern and non-brisk lymphoid infiltrate were associated with LVI (p=0.005 and 0.055, respectively). In our series, D2-40 detected LVI alone was not associated with metastasis, but intratumoral D2-40 detected LVI was associated with ~4X higher odds of metastasis to any site (p=0.035) and 3.5X greater odds of metastasis beyond the sentinel lymph node (p=0.037) compared to patients without intratumoral LVI.
In addition, tumors with invasion of vessels with a diameter >0.1mm and lesions with intratumoral LVI are each more likely to have invasion beyond skin (p= 0.016 and 0.003, respectively). Patients with MCPv+ MCC tend not to have central LVI (p=0.057).
Conclusions: D2-40 IHC increases the detection of LVI in PCMCC, and intratumoral D2-40 LVI is associated with adverse clinicopathologic parameters of outcome, in